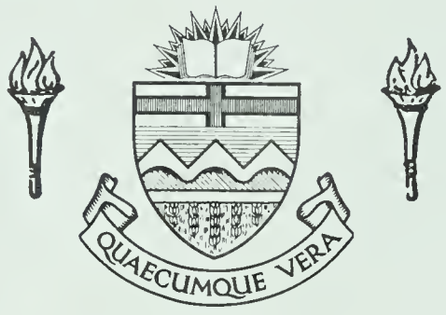


THE UNIVERSITY OF ALBERTA

A DIRECT COMPONENT OF COCAINE INDUCED SUPERSENSITIVITY TO NORADRENALINE

by

KANJI NAKATSU

A THESIS

SUBMITTED TO THE FACULTY OF GRADUATE STUDIES

IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE

OF MASTER OF SCIENCE

DEPARTMENT OF PHARMACOLOGY

EDMONTON, ALBERTA

July, 1968.

THE UNIVERSITY OF ALBERTA

FACULTY OF GRADUATE STUDIES

The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies for acceptance, a thesis entitled "A Direct Component of Cocaine Induced Supersensitivity to Noradrenaline", submitted by Kanji Nakatsu in partial fulfilment of the requirements for the degree of Master of Science.

ABSTRACT

The supersensitivity to noradrenaline, caused by cocaine, was investigated using the isolated rat vas deferens. Records of contractions were made on a kymograph with both isotonic and auxotonic writing arms being used; dose-response curves were obtained with both individual and cumulative dose administration of noradrenaline. Responses of the vas deferens were explained best by Paton's (1961) "rate hypothesis" of drug-receptor stimulation; on exposure to noradrenaline, the tissue responded with a rapid contraction (peak) followed by a relaxation to a steady state contraction (equilibrium). Dose-response curves for both peaks and equilibria assumed the typical sigmoid shape when noradrenaline was administered on an individual dose schedule but when they were obtained on a cumulative dose schedule the curve for peaks assumed the bell shape of autoinhibition while that for equilibria was sigmoid.

The supersensitivity due to cocaine caused a 10 fold, parallel shift of dose-response curves except for the portions of the cumulative dose curves at concentrations of noradrenaline greater than 2×10^{-6} g/ml. Since the slopes were expected to increase on the basis of a cocaine induced increase of intrinsic activity and expected to decrease on the basis of inhibition of uptake by cocaine, both mechanisms were implicated in explanation of cocaine supersensitivity.

In an attempt to destroy granular function and inhibit monoamine oxidase in the adrenergic nerves, rats were pretreated with reserpine and pheniprazine. Since tissues from these rats would be capable of

only a limited noradrenaline uptake, they could have been used to assess the place of inhibition of uptake in explanation of cocaine supersensitivity. However, the effect of reserpine to almost eradicate the equilibrium contractions did not allow analysis beyond that of untreated controls; reserpine because of this side effect is not a suitable agent for destruction of granules in the nerves of the rat vas deferens.

In tissues which were deprived of spare receptors by treatment with phenoxybenzamine, cocaine caused an increase of maximal responses to supramaximal concentrations of noradrenaline. Since cocaine produced increased maximal responses in these tissues on cumulative administration after a supramaximal dose of noradrenaline, it must act directly on the smooth muscle to cause potentiation.

Cocaine did not alter noradrenaline protection of alpha receptors against phenoxybenzamine; hence it appears unlikely that cocaine does affect the kinetics of drug-receptor interaction. The apparent increase in efficacy cannot be explained by an increase of dissociation rate constant (k_2) unless the kinetics of agonist- and antagonist-receptor interaction were affected in the same way.

Cocaine supersensitivity was decreased by treatment of the tissues with phenoxybenzamine which may have produced this effect by blocking the postsynaptic site of action of cocaine. However, since it has been shown that phenoxybenzamine inhibits uptake of noradrenaline, it is likely that uptake inhibition is responsible for part of the supersensitivity induced by cocaine.

Neither alteration of receptors nor inhibition of uptake satis-

factorily explains all the observations of cocaine induced supersensitivity to noradrenaline in this series of experiments, therefore, it is concluded that both actions of cocaine are involved in this phenomenon.

ACKNOWLEDGEMENTS

To my supervisor, Dr. R. J. Reiffenstein, I extend my appreciation for the direction and useful criticism which he gave during the course of this work.

I would also like to thank Dr. M. M. Vohra for his help and instruction given me in the initial stages of this project.

I wish to thank Mr. J. Lejderman for the assistance which speeded the completion of this work.

I would also like to acknowledge the support of the Alberta Heart Foundation.

Gifts of drugs were received from: Smith, Kline & French Inter-American Corporation, Lakeside Laboratories Inc., and Merck & Company Inc.

PUBLICATIONS

Parts of the work described in this thesis have been related earlier as follows:

Nakatsu, K., Vohra, M. M. & Reiffenstein, R. J. (1967). Contrast of Adrenergic Responses of Guinea Pig and Rat Vas Deferens. Can. Fed. Biol. Soc. Proc., 10, 138.

Nakatsu, K. & Reiffenstein, R. J. (1968). Increased Receptor Utilization: Mechanism of Cocaine Potentiation. Nature, Lond. 217, 1276-1277.

Reiffenstein, R. J. & Nakatsu, K. (1968). Increased Utilization of Adrenergic Receptors caused by Cocaine. Can. Fed. Biol. Soc. Proc. 11, 128.

To My Parents

TABLE OF CONTENTS

LIST OF FIGURES

INTRODUCTION

I. LITERATURE REVIEW

Although the adrenergic potentiating actions of cocaine have been known since 1910 (Frohlich & Loewi), the mechanism responsible for this phenomenon is still a subject of controversy. Over the last half century, theories advanced in an attempt to explain the phenomenon have been legion. However, these theories can be placed in two major categories;

A. inhibition of inactivation of catecholamines and

B. direct action of cocaine on the effector tissues.

A. INHIBITION OF INACTIVATION

The first of these categories is based on the hypothesis that cocaine produces a higher effective concentration of agonist drug at the receptor. If inactivation of the agonist were inhibited this would bring about an increased concentration of drug in the receptor region for a greater duration and would result in larger responses from the effector. This inhibition of inactivation might be accomplished in a number of ways.

a. Inhibition of Uptake into Nerves (Uptake Theory)

Burn (1932) first suggested the existence of catecholamine storage sites which could be replenished from the extracellular fluid adjacent to the nerve ending. Many investigators have shown that cocaine inhibits the uptake of catecholamines into the nerves, e.g. Farrant (1963) has shown that cocaine, methyl phenidate and pipradrol all compete with noradrenaline for the uptake mechanism

in nerves. Also well known is the supersensitivity to noradrenaline caused by these compounds. Since a strong correlation between inhibition of uptake and potentiation of responses to noradrenaline has been shown, it has been proposed that a causal relationship is involved. Direct support for this theory is not available but circumstantial evidence abounds. Muscholl (1961) showed excellent correlation between the reduction of uptake of noradrenaline, in rat heart, by cocaine and the increase in pressor activity.

Using dog hearts, Hardman, Mayer & Clark (1965) demonstrated that cocaine potentiated myocardial contraction better with noradrenaline than with adrenaline and not at all with isopropylnoradrenaline. They also found that cocaine inhibited the uptake of noradrenaline and adrenaline but not of isopropylnoradrenaline. More extensive work in this line has been completed by Trendelenburg (1965). Using optical isomers of noradrenaline and adrenaline, he has shown that those amines which are most readily taken up and therefore most likely to be affected by blockade of uptake are those which display the greatest potentiation. More precisely, the relative rates of uptake are l-noradrenaline > l-adrenaline > d-noradrenaline which is also the order of relative potentiation. Convincing evidence has been published by de la Lande & Waterson (1967) and de la Lande, Frewin, Waterson & Canell (1967) using perfused artery of the rabbit ear. The artery was mounted in an organ bath so that extralumenal and intralumenal fluids were kept separate. They postulated that since the noradrenergic nerves of

this tissue were outside the muscle sheath, differential exposure to drugs should elucidate the importance of "uptake theory." Hence, if uptake inhibition were responsible for potentiation then extralumenal exposure to noradrenaline and cocaine would result in greater potentiation than intralumenal exposure. This would be due to extralumenal (nerve side) exposure resulting in greater changes of inactivation of noradrenaline and thus greater changes in sensitivity. By their definition of sensitization (concentration of noradrenaline before cocaine divided by concentration after, which produces the same response) extralumenal exposure resulted in a sensitization of 9.2 and intralumenal exposure in a sensitization of 1.9. Additional support for explanation of supersensitivity by inhibition of uptake comes from potentiation of many sympathomimetics by various uptake inhibitors. (Trendelenburg, Muskus, Fleming & Gomez Alonso de la Sierra, 1962b; Thoenen, Huerliman & Haefely, 1964; Maxwell, Wastila & Eckhardt, 1966). Moreover, the relationship between denervation supersensitivity and the inability of denervated tissues to store noradrenaline has been explained by "uptake theory." (Trendelenburg et al, 1962b; Trendelenburg, 1966).

However, there are several instances in which this theory is inadequate, at least in its present form. For example, explanation of Trendelenburg's observations (1965), which showed that the order of inhibition and order of potentiation were the same, may be inappropriate in the light of recent work carried out in his laboratory and by others. Draskoczy (1967) and Draskoczy & Trendelenburg

(1968) found that differences in uptake between d- and l-noradrenaline were negligible. At concentrations from 1 ng/ml, which is in the range of uptake 1 (Iversen, 1965), to 100 µg/ml, which is in the range of uptake 2 (Iversen, 1965), they found no difference in rates of uptake of d- and l-noradrenaline. Cocaine (7.5 µg/ml) antagonized both isomers equally. Therefore, they concluded that "the uptake of noradrenaline through the neuronal membrane has little or no stereospecificity. Hence the stereospecificity of the sensitizing effect of cocaine can no longer be ascribed to the differences in the rate of uptake of the isomers." Draskoczy (1967) attributes the previous correlation between sensitization and inhibition of uptake (Trendelenburg, 1965) to an artifact of using equieffective doses of drugs. He speculates that the doses of l-noradrenaline used were in the range of the cocaine sensitive uptake 1 while the doses of d-noradrenaline used were in the range of the cocaine insensitive uptake 2 (Iversen, 1965). Barnett, Greenhouse & Taber (1968) showed a cocaine induced supersensitivity at concentrations of l-noradrenaline in the range of rapid uptake (uptake 2) which Iversen (1965) found to be affected only slightly if at all by cocaine. Therefore an explanation based on inhibition of uptake cannot satisfy these observations if the uptake properties of rat heart and vas deferens are similar.

Maxwell (1965) points out that in an isolated organ bath the quantity of agonist is so large that an equilibrium between nerve and bath concentrations will be attained and therefore explanation

by "uptake theory" is inadequate. Here, "uptake theory" could be valid only if the rate of uptake from the biophase (K_b) were approximately equal to the rate of diffusion into the biophase (K_d). He provided a mathematical model to show that if K_b were much larger than K_d or vice-versa then inhibition of uptake could have only a slight effect on the drug concentration in the biophase. He also introduced preliminary evidence that reduction of uptake and binding does not correlate well with the increase in responses of rabbit aortic strips to noradrenaline. It was demonstrated that cocaine, guanethidine and methyl phenidate all produced both supersensitivity to noradrenaline and reduction of the binding rate of labelled noradrenaline, in rabbit aortic strips, (Maxwell et al, 1966). Yet it was found that the increase in responses did not correlate with the decrease in binding when actual curves were compared to theoretical curves derived from a simple uptake model. Both slopes and threshold values differed. For example, methyl phenidate produced a decrease in binding without a concomitant increase in response. The possibility that this is due to a threshold effect is unlikely because the reverse effect has also been reported: Crout, McAnelly & Tatum (1967) found that low concentrations of cocaine hydrochloride between 0.9×10^{-7}M and 0.9×10^{-6}M (0.03 to 0.3 µg/ml) produced small but significant leftward shifts in the dose-response curve to l-noradrenaline. These concentrations produced no inhibition of uptake of l-H^3-noradrenaline.

Maxwell et al (1966) demonstrated that after exposure of a tissue to guanethidine, the inhibition of uptake and binding could be washed

out by changing the bathing fluid. However, supersensitivity still remained after the uptake effects had disappeared, therefore the effects on uptake and sensitization, in this case, probably were dissociated.

b. Inhibition of Monoamine Oxidase

Gaddum and Kwiatowski (1938) introduced the hypothesis that cocaine caused supersensitivity to adrenaline by the inhibition of monoamine oxidase; that is, inhibition of metabolism resulting in increased concentrations of agonist which would lead to potentiated responses. They showed that both cocaine and ephedrine sensitized the perfused rabbit ear, cat nictitating membrane and frog heart; also both drugs could inhibit monoamine oxidase. The denervation studies of Burn & Robinson (1952) supported this hypothesis. They found that denervation sensitized the nictitating membrane, blood vessels and iris to noradrenaline. Concurrent with this was a decrease in the amount of monoamine oxidase. While the above evidence provides good support for the hypothesis more recent work renders the hypothesis unacceptable.

Philpot (1940) required concentrations of cocaine 1,000 to 1,000,000 times those required to produce supersensitivity in order to inhibit the enzyme. Furthermore, cocaine produces supersensitivity to cobefrine yet this substance is not metabolized by monoamine oxidase (Jang, 1940). More convincing evidence in opposition to the monoamine oxidase hypothesis came with the advent of potent monoamine oxidase inhibitors which showed little or no potentiating activity

(Furchgott, 1955).

c. Inhibition of Catechol-O-Methyl Transferase

With the discovery of catechol-O-methyl transferase in 1957, by Axelrod, this enzyme was suspected of involvement in cocaine supersensitivity. The basis for this was the same as for monoamine oxidase inhibition. However, this hypothesis was quickly found to be untenable. First, cocaine produced supersensitivity to amines not metabolized by catechol-O-methyl transferase (Trendelenburg et al, 1962a, b); secondly, inhibitors of this enzyme produced prolongation of responses to noradrenaline and adrenaline but little or no increase in magnitude (Wylie, Archer & Arnold, 1960); and thirdly, cocaine has not yet been shown to inhibit this enzyme (Wylie et al, 1960).

B. DIRECT ACTION OF COCAINE ON THE EFFECTOR TISSUES

Since uptake and metabolic theories have not been found to be without shortcomings, a second category has been presented. It is possible that the potentiating activity of cocaine is mediated through a mechanism on or in the effector cell. Possibly cocaine supersensitivity results from a secondary change of the muscle cell caused by a postsynaptic action of cocaine.

a. Alteration of Receptors (Alteration Theory)

In 1937, Clark suggested that potentiation due to cocaine might be the result of a change at the receptor. At this time there was no evidence to support this hypothesis but recently more support has been shown. Maxwell (1965) has shown that methyl phenidate, guanethidine and cocaine all potentiate the actions of noradrenaline and

suggested the existence of a common mechanism. In 1961 he demonstrated the ability of methyl phenidate to antagonize the blocking action of phentolamine on the cat nictitating membrane and perfused hind limbs. Therefore it was thought that methyl phenidate acted directly on the muscle. Using a series of mathematical equations based on those of Clarke he calculated that methyl phenidate probably increased the affinity between noradrenaline and its receptors on the muscle cells. Maxwell (1965) also found that methyl phenidate protected the alpha adrenergic receptor against the irreversible blocking agent, phenoxybenzamine. Considering supersensitivity at a molecular level he stated:

> "It is speculated that guanidines may change the conformation of the receptor 'surface' in such a way as to improve hydrogen bonding or ion pair formation with selected amines and hence to enhance the affinity of receptor-amine complex."

Karr and Innes (1966) showed that noradrenaline and phentolamine could protect against the effects of cocaine in cat spleen strips. Since protection was not afforded by histamine, acetylcholine, isopropylnoradrenaline or pronethalol, they concluded that cocaine must act at or near the alpha adrenergic receptor. Tyramine in doses which acted presynaptically but not postsynaptically could not protect against the effects of cocaine, which, they concluded, ruled out a presynaptic action.

Bevan and Verity (1967) produced a nerve free preparation by stripping rabbit aortic strips of adventia. They found that in

these acutely denervated preparations cocaine did not change the slope of or shift the dose-response curve but did significantly increase (1.43 times) the maximum contraction of the strips. Since cocaine (3 µg/ml) did not produce a significant shift of the dose-response curves in denervated but did in control strips they conclude that the supersensitivity produced was due to both a presynaptic mechanism and a direct action on the smooth muscle. Furthermore, Barnett et al (1968) have shown a cocaine induced increase in the maximal responses of the rat vas deferens to cumulative doses of noradrenaline; this is best explained by an increase in intrinsic activity due to the presence of cocaine. The molecular changes induced by cocaine to cause this supersensitivity may be the same as those involved in denervation supersensitivity, (Trendelenburg et al, 1962b).

Additional evidence has been provided by Reiffenstein (1968) on cat spleen strips. He demonstrated that while cocaine did not increase the rate of contraction it increased the strength of contraction induced by noradrenaline. On the basis of "uptake theory" the increase in effective concentration should have increased contraction rate. Therefore an explanation based on an alteration of receptor kinetics using Paton's (1961) "rate theory" was proposed. There are other reports of amines which are not taken up but nevertheless still potentiated by cocaine. Nickerson & Kalsner (personal communication) found potentiation in rabbit aortic strips on cumulative administration of cocaine to tissues already exposed to methoxamine, which is neither taken up nor metabolized. Leszkovszky &

Tardos (1968) have shown that cocaine potentiated the responses of isolated cat spleen strips to isopropylnoradrenaline, an amine not taken up by adrenergic nerves (Hardman et al, 1965). The same supersensitivity to isopropylnoradrenaline in the presence of cocaine has been demonstrated by Davidson & Innes (1968).

Although much of the evidence in support of "alteration theory" is convincing, some of it also supports other theories. Experiments by Karr & Innes (1966) which showed that phentolamine protected against the actions of cocaine could simply have been due to the alpha receptor blocking activity of phentolamine. Maxwell's (1965) protection studies can also be explained by "uptake theory"; since methyl phenidate inhibits uptake of noradrenaline (Maxwell, 1965) it could have increased the concentration of noradrenaline in the receptor region sufficiently that noradrenaline was the real protector.

b. Interruption of Natural Desensitization

The association between denervation supersensitivity and cessation of noradrenaline leakage due to degeneration of nerves led Fleckenstein and Bass (1953) to hypothesize that continuous stimulation of a tissue by spontaneously released noradrenaline maintained that tissue in a state of desensitization. They reasoned that when stimulation is stopped, the muscle frees itself of desensitization and becomes more sensitive. Therefore cocaine as a local anesthetic could act in this manner by inhibiting the release of noradrenaline from the nerves. However, there are a number of objections to this explanation of the action of cocaine. The concentrations of cocaine

(1 µg/ml) which produce supersensitivity are below the threshold of local anesthesia (Andersen & Gravenstein, 1966). All other local anesthetics do not produce supersensitivity, (Muscholl, 1961). Furchgott (1955) showed that rabbit aortic strips were sensitized even after long exposure to noradrenaline and adrenaline. He exposed tissues to these agonists for periods up to 30 minutes, on addition of cocaine at this point, the strips contracted further. Potentiation in this case cannot be explained by a release from chronic desensitization.

C. PRESENT STATUS OF PROBLEM

At this juncture it would appear that the "uptake theory" and "alteration theory" claim the most support. Although it seems most pharmacologists belong to either one or other of these schools of thought, it has never been established that these theories are mutually exclusive. Rather it seems more logical to compare cocaine to noradrenaline. Since noradrenaline has many sites of action it is not unlikely that cocaine would fit more than one of these sites, e.g. Vohra (1968) demonstrated that cocaine, in high concentrations, could directly stimulate alpha receptors.

II. STATEMENT OF PROBLEM AND THEORETICAL BASIS FOR EXPERIMENTS

The purpose of this project was to provide evidence for a direct sensitizing effect of cocaine and to provide a working model at the molecular level.

If the noradrenaline stores in nerves of a tissue can be made saturable then this tissue could be used to test the "uptake theory" of cocaine supersensitivity. In a tissue whose noradrenaline stores are saturated, supersensitivity to cocaine would not be predictable from "uptake theory" because cocaine could not increase the concentration of agonist in the receptor region by inhibition of uptake. In order to prepare tissues with saturable, noradrenaline storage depots both destruction of granular function and inhibition of monoamine oxidase would be required (Furchgott, Kirpekar, Rieker & Schwab, 1963). Van Orden, Bloom, Barrnett & Giarman (1967) have shown that reserpine can be used to eliminate the large granular storage sites for noradrenaline since it can prevent granular accumulation of noradrenaline. On the other hand a monoamine oxidase inhibitor can be used to prevent loss of intracellular noradrenaline by metabolism (Axelrod, 1966). After these two major fates of noradrenaline are blocked the nerves should have a limited capacity for uptake of noradrenaline; indeed Van Orden et al (1967) found that after reserpine and iproniazid treatment nerves could only accumulate noradrenaline in the extravesicular space.

Phenoxybenzamine, an irreversible alpha adrenergic receptor blocking agent, can be used to reduce the number of effectual receptors

in an isolated preparation (Nickerson, Henry & Nomaguchi, 1953). If a sufficient number of receptors were blocked the maximal response would be restricted by the number of receptors and their intrinsic activity (Ariens, 1964) rather than by the contractile limitations of the muscle. Therefore, any increase in the maximal response, to a supramaximal concentration of noradrenaline, in the presence of cocaine can be attributed to a direct effect on the effector tissue at or near the receptor.

On the basis of the "occupation hypothesis" of drug-receptor interaction a direct action of cocaine to produce supersensitivity can be explained as an increase in the intrinsic activity (Ariens, 1964) or efficacy (Stephenson, 1956). An increase in drug-receptor affinity would also provide an adequate explanation. On the other hand, an explanation in terms of Paton's (1961) "rate hypothesis" could be appropriate. An increase in the rate of dissociation (k_2) would lead to a greater number of receptors free to combine with agonist and to a potentiation of response. If either affinity or dissociation was altered by cocaine any change should be shown as an increase or decrease in the protection afforded the alpha receptors, by a high concentration of noradrenaline, against phenoxybenzamine (Furchgott, 1964). If cocaine were to increase noradrenaline-receptor affinity, phenoxybenzamine should have a reduced opportunity to block alpha receptors. Hence responses of tissues after partial alpha receptor blockade in the presence of noradrenaline with cocaine would be predicted to be greater than those from tissues exposed to phenoxybenzamine in the presence of noradrenaline but not cocaine. However, if cocaine induced

an increase in dissociation phenoxybenzamine should have an increased opportunity to block alpha receptors and therefore responses would be expected to be opposite to those predicted if cocaine caused an increase of affinity. Furthermore, an increase in dissociation (k_2) should increase the fade ratio (f), where

$$f = \frac{k_2}{k_1 x + k_2} \quad \text{or} \quad \frac{\text{equilibrium}}{\text{peak}}$$

k_1 = association constant

x = concentration of agonist (Paton, 1961).

The significance of uptake inhibition in cocaine supersensitivity might be demonstrated using the irreversible uptake inhibitor phenoxybenzamine (Gillespie, 1965; Furchgott, 1966). If uptake inhibition plays a role in cocaine supersensitivity, irreversible blockade of some uptake sites before addition of cocaine should reduce the sensitization by cocaine because cocaine would not be able to cause as large a reduction in inactivation of agonist. Hence, if sensitization (shift of dose-response curve) is reduced in phenoxybenzamine treated tissues compared to untreated controls, "uptake theory" would be supported.

METHODS AND MATERIALS

I. MATERIALS

A. Apparatus

The apparatus is illustrated in Figure 1. The 10 ml muscle bath unit was designed so that bathing solution would be prewarmed while passing through the warming coil. The water jacket enclosing both the muscle bath and warming coil was maintained at 37°C by continuous circulation of water, from a reservoir, whose temperature was thermostatically controlled. Krebs solution from a reservoir passed into the unit where its level and flushings were controlled by pinchcocks. Both the Krebs reservoir and muscle bath were aerated with O_2 95% and CO_2 5%; aeration of the bath was effected through a fine polyethylene tubing (Clay-Adams PE20) passed through the drain opening of the muscle bath.

The prostatic end of the vas deferens was anchored in the muscle bath to a glass tissue holding rod while the epididymal end was connected to the writing arm. Tissue contractions were magnified 7 times and recorded on smoked kymograph paper moving 0.01 mm/sec.

In practice 4 units with tissues from 2 animals were accommodated by using a Palmer kymograph extension. When isotonic recordings were made levers were adjusted so that the tension on the tissues was 0.5 g. When auxotonic (Paton, 1957) recordings were made, the base line tension was 0.3 g and maximal tension was about 0.6 g.

B. Preparation of Vasa Deferentia

Male albino rats (Wistar and Sprague-Dawley strains) weighing

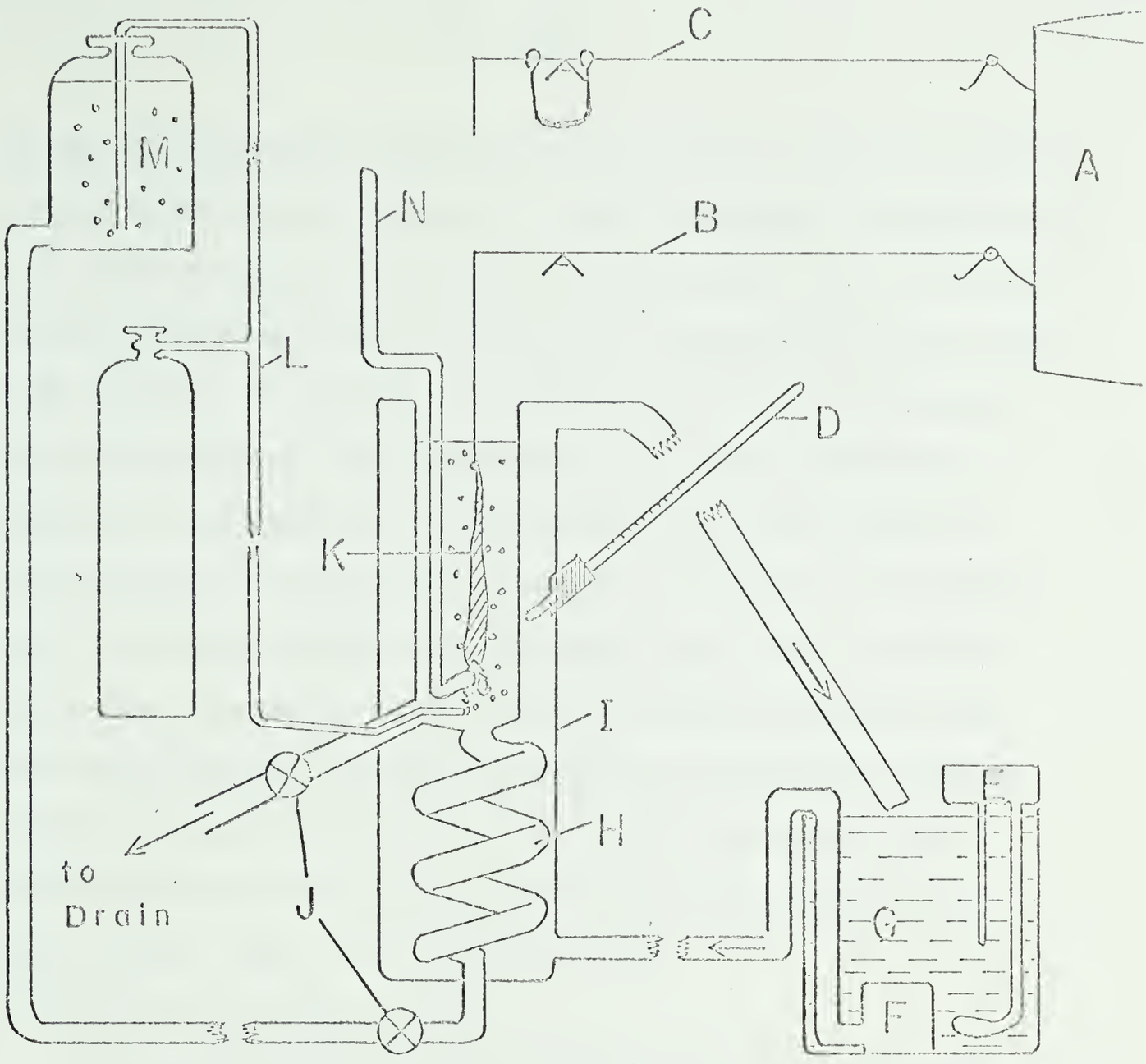


Figure 1. Apparatus.

A. Kymograph
B. Isotonic Writing Arm
C. Auxotonic Writing Arm
D. Thermometer
E. Thermostat Controlled Heater
F. Pump
G. Water Reservoir
H. Warming Coils
I. Water Jacket
J. Stopcocks
K. Vas Deferens
L. Aeration Unit
M. Krebs Solution Reservoir
N. Tissue Holding Rod

between 200 g and 225 g were killed by stunning and cervical fracture. The vasa deferentia were exposed by a midline incision and pushing the testes from the scrotum into the peritoneal cavity. They were then removed quickly and gently and placed in a shallow dish, filled with Krebs solution, for dissection. Connective tissue and superficial vasculature were cut away with the aid of a 4-power illuminating magnifier. The tissues were then mounted in the muscle baths as shown in Figure 1 and allowed to relax and equilibrate for 1 hour at base line tensions before being subjected to any tests. In this period they were washed every 10 minutes in order to assure a constant bathing medium. After this treatment, reproducible responses could be elicited for up to 16 hours provided the tissues were washed regularly and were not exposed to high concentrations of drugs for more than a few minutes at a time.

C. Preparation of Krebs Solution

Two stock solutions were made, from which daily requirements of Krebs solution could be made. Stock A consisted of: NaCl, 76.3 g; KCl, 3.9 g; $MgCl_2.6H_2O$, 2.63 g; $Na_2HPO_4.H_2O$, 1.83 g; $CaCl_2.2H_2O$, 4.1 g; in 1000 ml of distilled and deionized water. Stock B consisted of 2.6% $NaHCO_3$. The solution employed daily was made from 100 ml Stock A, 80 ml Stock B, 10 g destrose and 950 ml distilled and deionized water, which then had the following composition: Na^+, 138.5 mM; K^+, 4.36 mM; Ca^{++}, 2.47 mM; Cl^-, 127.4 mM; HCO_3^-, 21.9 mM; $H_2PO_4^-$, 1.16 mM; glucose 49.2 mM.

D. Preparation of Drugs

Stock solutions of drugs were prepared from which daily requirements could be made by dilution in Krebs solution. Dissolution of drugs was effected by the procedures outlined in the Merck Index of Chemicals and Drugs (6th edition). Drugs were obtained as follows:

a. Cocaine hydrochloride, British Drug Houses (Canada) Ltd.

b. Phenoxybenzamine hydrochloride, Smith, Kline and French Inter-American Corporation.

c. Pheniprazine (JB-516), Lakeside Laboratories Inc.

d. Noradrenaline bitartrate, Winthrop Laboratories.

e. Methacholine chloride, Merck and Company Inc.

f. Reserpine, Ciba Company Ltd.

Drug concentrations are expressed in terms of the salts.

II. METHODS

A. Construction of Dose-Response Curves

Dose-response curves in this section were produced using both single dose and cumulative dose administration of noradrenaline. Furthermore, both isotonic and auxotonic levers were employed. When single doses were used the tissue was allowed to attain a steady state contraction, which usually took from 5 to 7 minutes, then the tissue was washed and allowed to rest for twice the duration of the exposure. When cumulative doses were used, the tissue was allowed to reach a steady state contraction before each subsequent dose of noradrenaline.

a. Normal Responses

The responses of the vas deferens were characterized under isotonic and auxotonic conditions using single and cumulative noradrenaline administration. The usual pattern of agonist concentration was 1, 4, 16, 64, 256 and 1024 X 10^{-7} g/ml. After obtaining these dose-response curves to noradrenaline alone, the tissues were given sufficient time to recover (1/2 to 1 hour). Then the dose-response curves were repeated in the presence of cocaine (10^{-6} g/ml). Cocaine was added one minute prior to each dose of noradrenaline when single doses were used and one minute prior to the first dose when cumulative doses were used, this was the minimum time which allowed cocaine to exert its full action. The usual pattern of noradrenaline concentration in the presence of cocaine was 0.06, 0.25, 1, 4, 16, 64, 256 and 1024 X 10^{-7} g/ml.

b. Saturation of Noradrenaline Storage Depots

In an attempt to produce saturable noradrenaline storage depots, rats were pre-treated with reserpine (2 days, 5 mg/kg, S.C.) to destroy granular stores, and with pheniprazine (1 day, 10 mg/kg, S.C.) to inhibit monoamine oxidase. Both auxotonic recordings with cumulative doses and isotonic recordings with single doses were made with tissues from pre-treated rats. Recordings in both cases involved a dose-response curve in the absence of cocaine, followed by a recovery time (1/2 to 1 hour) and a dose-response curve in the presence of cocaine (10^{-6} g/ml).

c. Effects of Pheniprazine or Reserpine

The effects of pheniprazine or reserpine alone were investigated. Rats were pre-treated with pheniprazine (1 day, 10 mg/kg, S.C.) or reserpine (1 day, 5 mg/kg, S.C.) and dose-response curves, both in the absence of and presence of cocaine (10^{-6} g/ml), were obtained on an isotonic, single dose basis.

B. Reduced Receptor Concentration

In order to produce a tissue with a limited portion of alpha receptors, the irreversible alpha blocking agent, phenoxybenzamine was used. The tissues were mounted on the isotonic recording apparatus and allowed to equilibrate for 1¼ hours, at this time a maximal response (noradrenaline 10^{-4} g/ml) was obtained as a reference. After washing, the tissues were allowed to relax for 15 minutes, then were subjected to phenoxybenzamine (5×10^{-9} g/ml) for 3 minutes. The phenoxybenzamine was then washed out of the bath and tissues were washed at 10 minute intervals for 1 hour. Experiments then proceeded as follows.

a. Tissues were subjected to a high concentration of noradrenaline (10^{-4} g/ml) for 6 minutes which allowed it to attain a steady contraction. After another hour of rest and regular washing the same procedure was repeated. Another hour of rest and washing followed, then the tissues were subjected to the same procedures except that cocaine (10^{-6} g/ml) was added to the baths 1 minute before the noradrenaline.

b. Tissues were subjected to a high concentration of noradrenaline (10^{-4} g/ml) for 6 minutes. Then noradrenaline was added cumulatively

to increase the concentration in the bath to 2 X 10^{-4} g/ml and tissues were left for a further 6 minutes. At this time cocaine (10^{-6} g/ml) was added cumulatively and tissues were left for a further 6 minutes.

c. Methacholine Controls

To ensure that cocaine supersensitivity was specific, effects of cocaine on a non-adrenergic response were studied. Responses 1 minute in duration, were elicited at 15 minute intervals. Two responses to methacholine (10^{-4} g/ml) alone and two to methacholine, in which cocaine was added one minute before, were obtained.

C. Protection Experiments

Tissues were exposed to a protective concentration of noradrenaline (10^{-4} g/ml) for 6 minutes which allowed them to attain a steady state contraction. Then cumulatively cocaine (10^{-6} g/ml) was added, 1 minute later phenoxybenzamine (5 X 10^{-8} g/ml) was added cumulatively. After phenoxybenzamine exposure for 3 minutes the tissues were washed and rested for 1 hour after which their maximum responses to noradrenaline (10^{-4} g/ml) were elicited. Controls for these tissues were contralateral partners treated in the same manner except with cocaine (10^{-6} g/ml for 4 min) following the washout of phenoxybenzamine rather than preceding the addition of phenoxybenzamine.

D. Inhibition of Uptake

Maximum responses were elicited from the tissues by exposure to noradrenaline (10^{-4} g/ml). Then the tissues were washed and allowed to relax for 15 minutes; then they were exposed to phenoxybenzamine (5 X 10^{-9} g/ml) for 3 minutes to inhibit uptake of noradrenaline.

After washing and resting for one hour dose-response curves were obtained on the basis of a 5 minute cycle and isotonic recording. Tissues were exposed to noradrenaline for 1 minute followed by 4 minutes of rest and washing. Following this, dose-response curves were obtained in the presence of cocaine; a 6 minute cycle was used. Tissues were exposed to cocaine for 1 minute, then noradrenaline was added cumulatively and left in the bath for one minute. At this time the tissues were washed and allowed to rest for 4 minutes before the subsequent exposure to cocaine and noradrenaline. Controls were contralateral partners in which the only difference was omission of phenoxybenzamine treatment.

E. Statistics and Plotting Procedures

All responses represented graphically were calculated as the mean of the percentage of the maximum response that the tissue was capable of producing. Standard errors of the mean were obtained from the formula:

$$S.E._{\bar{x}} = \sqrt{\frac{\sum x^2 - \frac{(\sum x)^2}{n}}{n(n-1)}}$$

Tests of significance of difference were effected using Student's t-test for paired data, where

$$t = \frac{\sum \frac{(x_1 - x_2)}{n}}{\sqrt{\frac{\sum (x_1 - x_2)^2 - \frac{(\sum x_1 - \sum x_2)^2}{n}}{n(n-1)}}}$$

Values of probability were obtained from statistical tables by Fisher & Yates (1957).

RESULTS

A. DOSE-RESPONSE CURVES

All graphs in this section are constructed with the logarithm of the concentration of noradrenaline on the abscissa and the response as a percentage of maximum on the ordinate. Points represent the mean of values from 6 rats and vertical bars represent the standard error of the mean.

a. Normal Responses of Vas Deferens

Figure 2 shows typical responses to graded (increased by multiples of 4) single doses of noradrenaline using isotonic or auxotonic levers. The initial rapid contraction of the vas deferens is shown on the record as a fast rise (peak). After this peak contraction, the tissue relaxed to a steady state (equilibrium). The dose-response curves with the isotonic lever (Figure 3) differ from those with the auxotonic lever (Figure 4) only because the smaller responses are magnified in auxotonic recordings. Cocaine in all cases produced a 1 cycle leftward shift on the logarithm of dose scale. The maximal equilibria were unchanged by cocaine. The records obtained with cumulative administration of noradrenaline (Figure 5) were similar to the single dose records at low concentrations. However at high concentrations, the magnitude of the rapid rise above the previous equilibrium was progressively reduced. Hence a bell shaped dose-response curve was obtained for peaks and a sigmoid curve for equilibria (Figures 6 & 7). The effect of cocaine was to increase the equilibria at low concentrations of noradrenaline while depressing at high concentrations. The peaks were shifted leftward

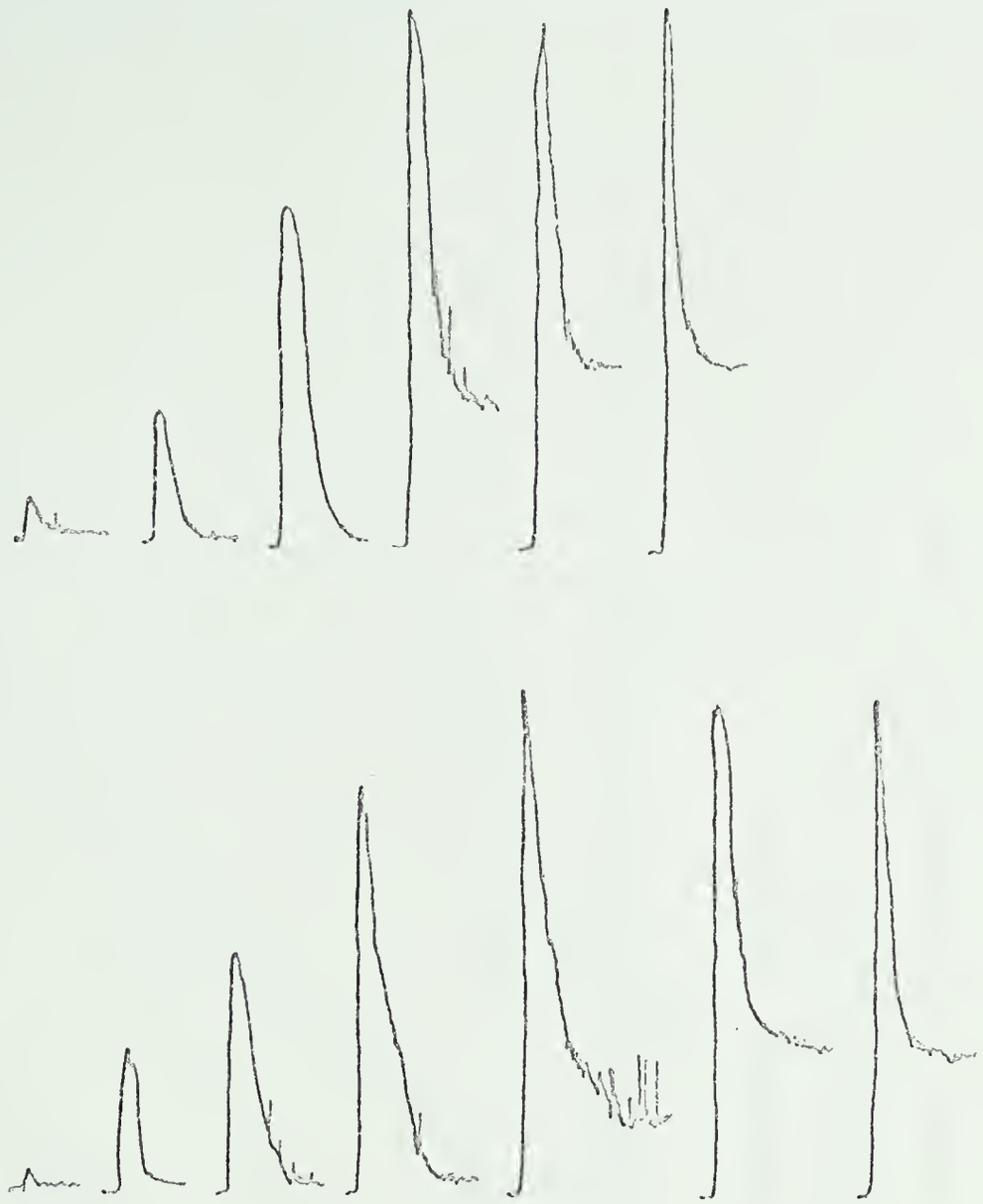


Figure 2. Tracings of Responses of Vas Deferens to Single Doses of Noradrenaline.

Upper Tracing: Responses to 1, 4, 16, 64, 256 and 1024 X 10^{-7} g/ml noradrenaline.

Lower Tracing: Responses to 0.06, 0.25, 1, 4, 16, 64 and 256 X 10^{-7} g/ml noradrenaline. Cocaine (10^{-6} g/ml) was added one minute before each response.

1 cm = 6 min.

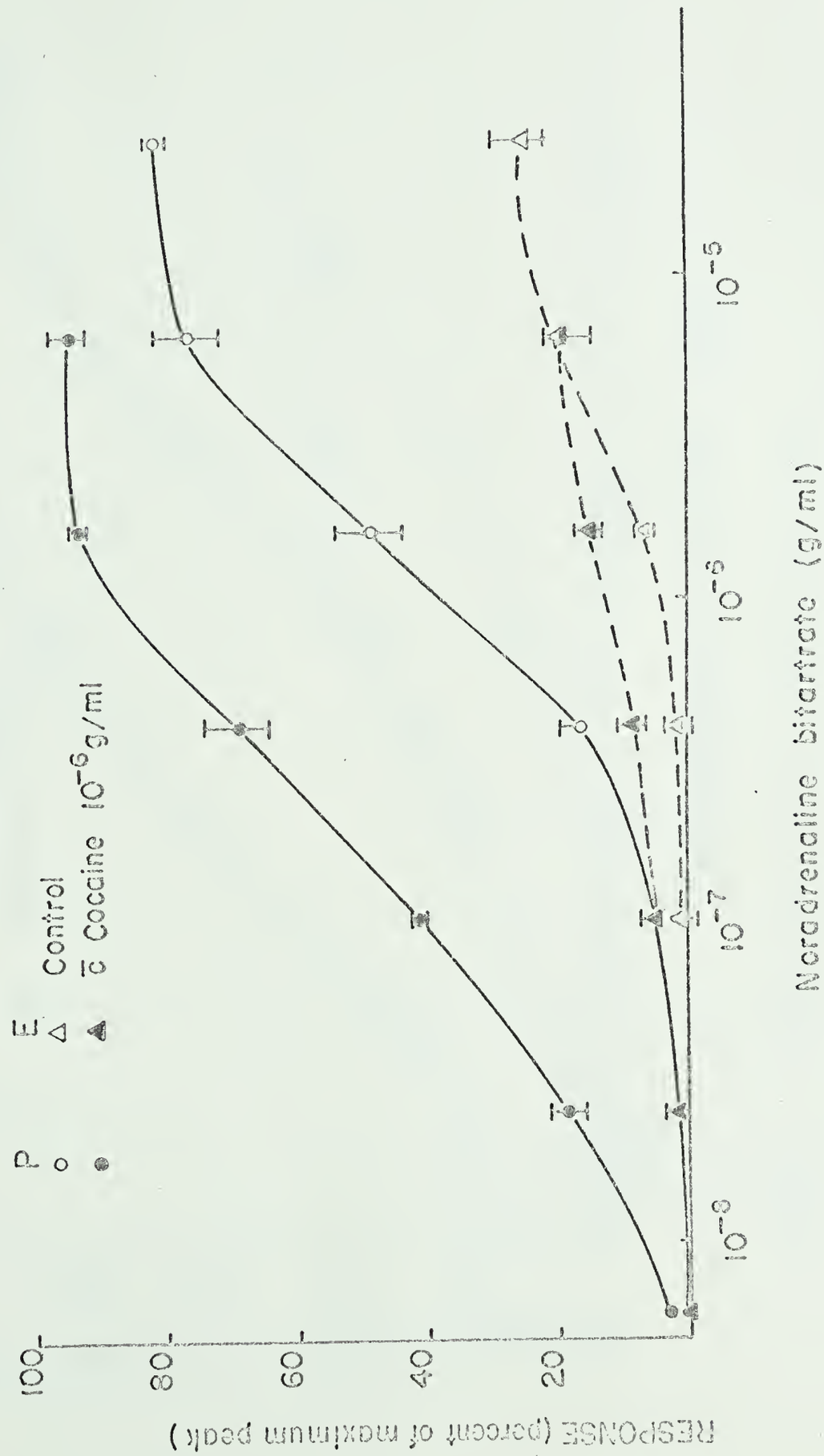


Figure 3. Single Dose, Isotonic Dose-Response Curves.

Points represent the means of values obtained using isotonic levers and single doses of noradrenaline.

In this as in subsequent figures, P = Peak and E = Equilibrium phase of response.

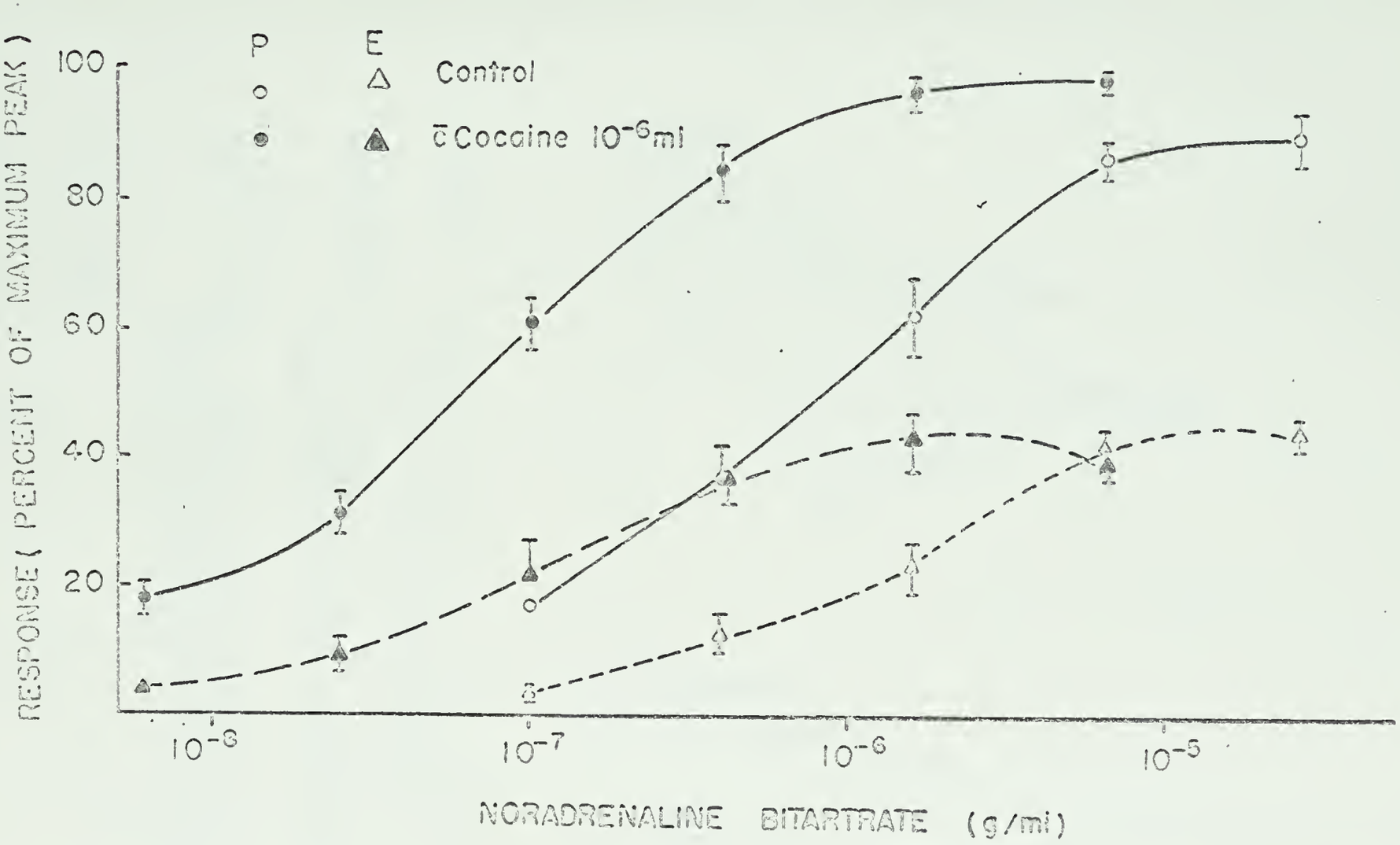


Figure 4. Single Dose, Auxotonic Dose-Response Curves.

Points represent the means of values obtained using auxotonic levers and single doses of noradrenaline.

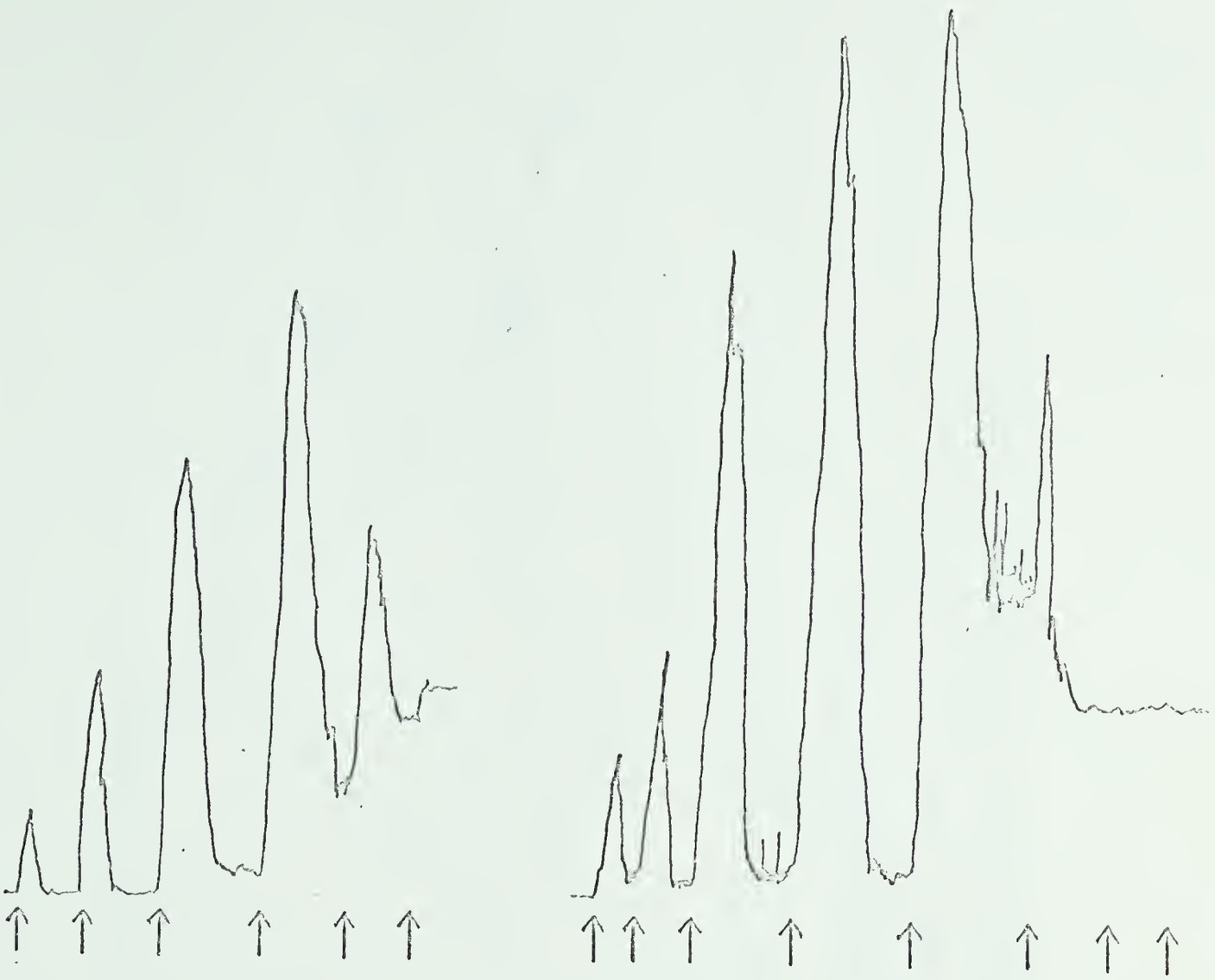


Figure 5. Tracings of Responses of Vas Deferens to Cumulative Doses of Noradrenaline.

Left Tracing: Responses to 1, 4, 16, 64, 256, and 1024 X 10^{-7} g/ml noradrenaline administered cumulatively.

Right Tracing: Responses to 0.06, 0.25, 1, 4, 16, 64, 256 and 1024 X 10^{-7} g/ml noradrenaline administered cumulatively in the presence of cocaine (10^{-6} g/ml).

1 cm = 5 min.

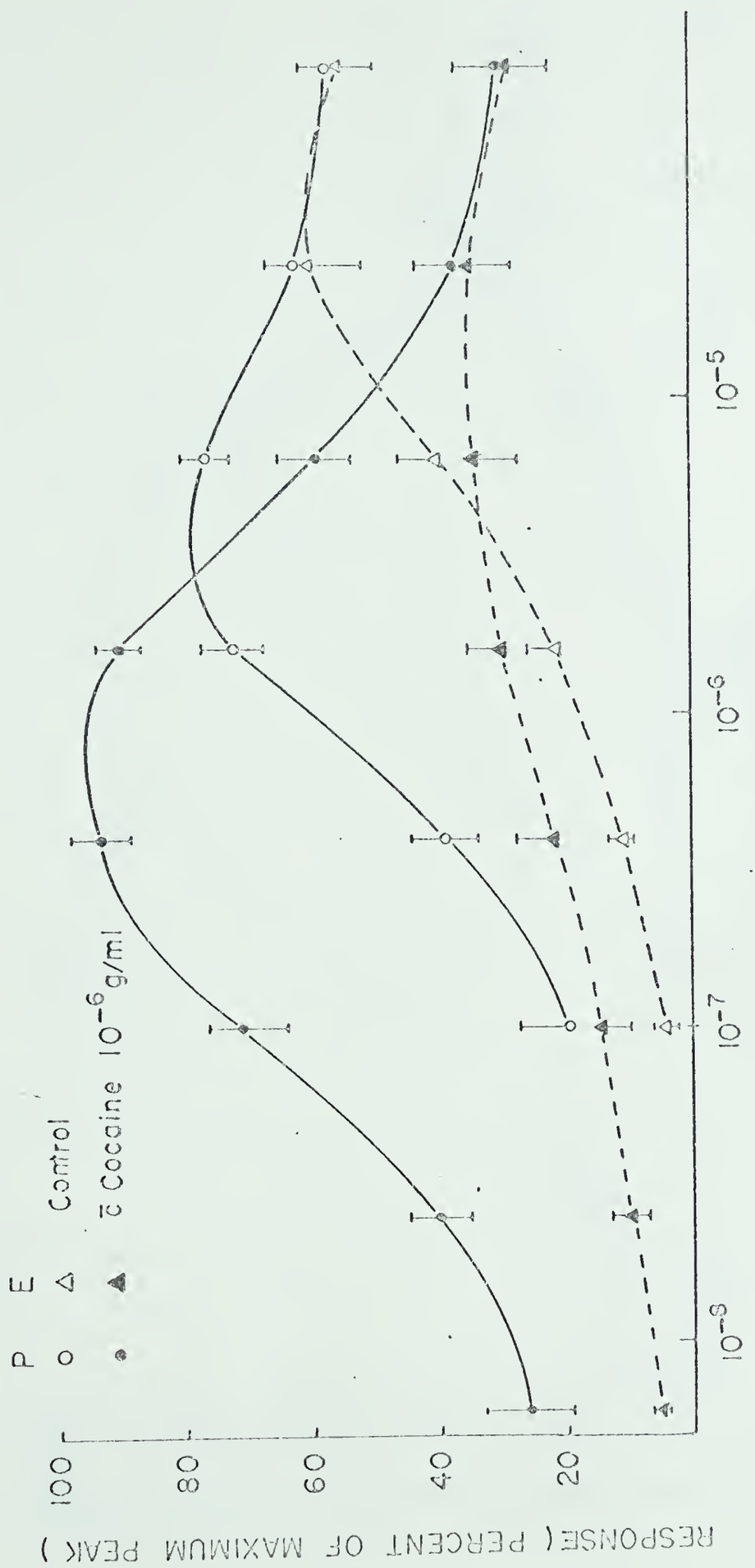


Figure 6. Cumulative Dose, Auxotonic Dose-Response Curves.

Points represent the means of values obtained using auxotonic levers and cumulative doses of noradrenaline.

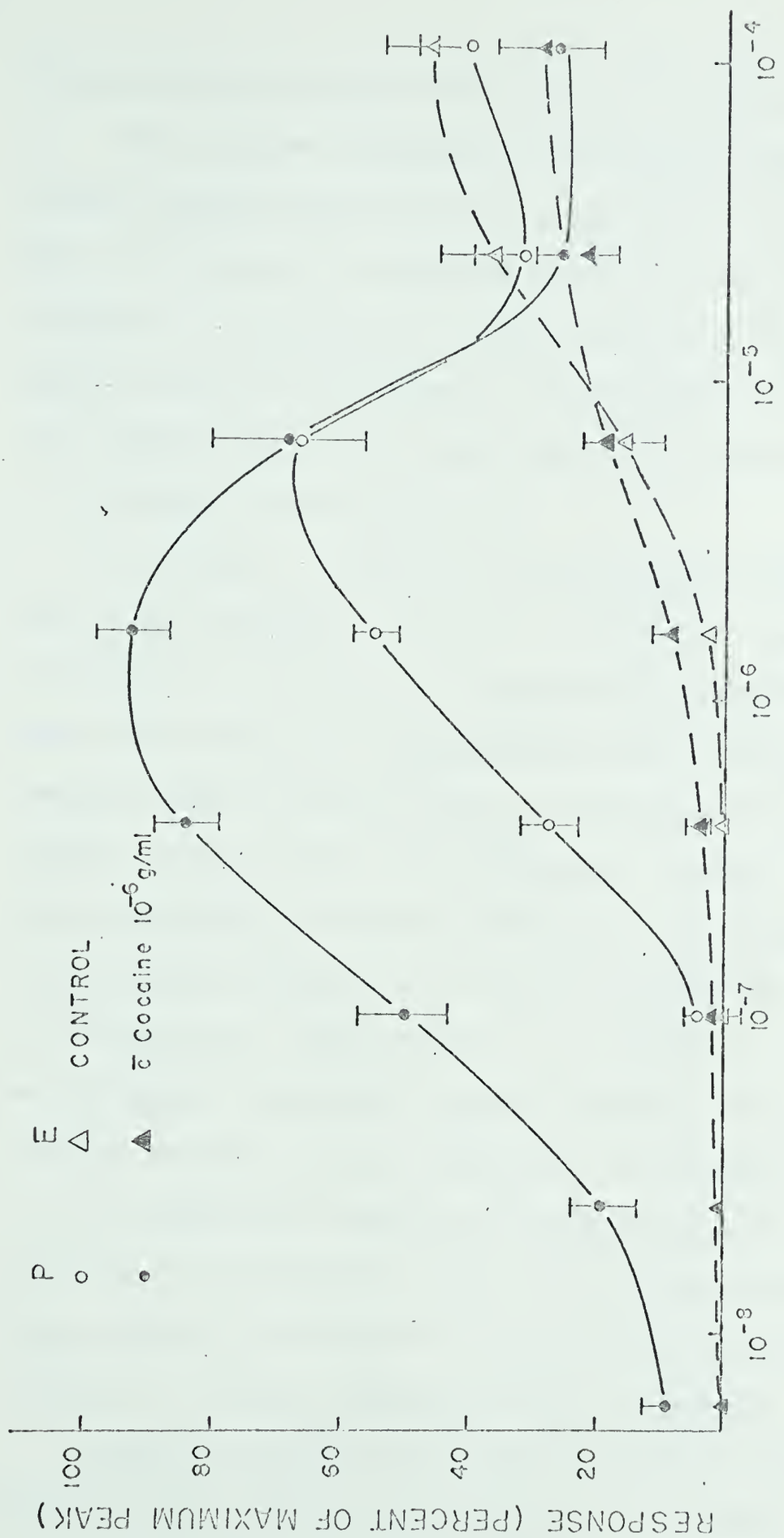


Figure 7. Cumulative Dose, Isotonic Dose-Response Curves.

Points represent the means of values obtained using isotonic levers and cumulative doses of noradrenaline.

1 cycle and their maxima were increased by cocaine.

Fade-ratio was calculated as equilibrium divided by peak using values obtained from single dose, auxotonic dose-response curves (Figure 4). The presence of cocaine had no effect on values of fade-ratio obtained by extrapolation of linear portions of the curves. For example, fade-ratio calculated at a noradrenaline concentration of 2 X 10^{-6} g/ml was 0.42 in the control and 0.44 in the presence of cocaine.

b. Saturated Noradrenaline Stores

The effects of reserpine and pheniprazine on isotonic, single dose curves are shown in Figure 8. The shift of the curve for peak contractions is the same as in untreated controls (Figure 3); pretreatment with reserpine and pheniprazine did not have the effect of reducing cocaine supersensitivity as predicted by "uptake theory." Cocaine did not increase the maximum peak response, however the equilibrium responses were almost eliminated by pretreatment. In dose-response curves obtained in an auxotonic cumulative dose manner (Figure 9) the equilibria were depressed by pretreatment but not as severely as in isotonic recordings. Cocaine produced a 10 fold sensitization for peak responses and also gave rise to an increase of maximum peak. If the noradrenaline storage depots became saturated after the first few doses of noradrenaline, neither of these observations would have been predicted on the basis of "uptake theory" and therefore another mechanism for cocaine supersensitivity is suggested.

Isotonic recordings with single dose administration of noradrenaline were used in testing the effects of pretreatment with reserpine

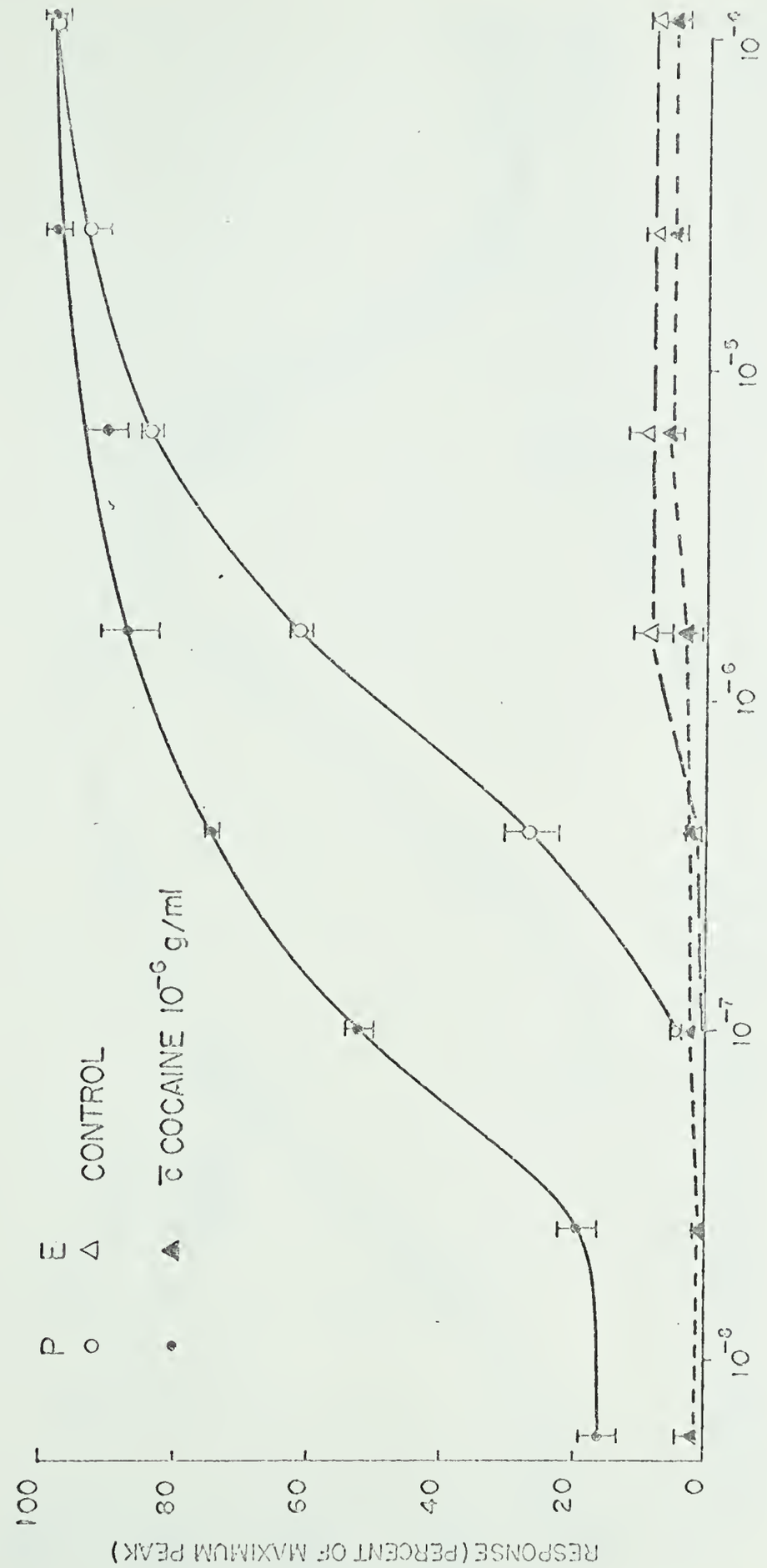


Figure 8. Single Dose, Isotonic Dose-Response Curves After Reserpine and Pheniprazine Pretreatment.

Points represent the means of values obtained using isotonic levers and single doses of noradrenaline. Animals were pretreated with reserpine (5 mg/Kg, I.P., 2 days) and pheniprazine (10 mg/Kg, I.P., 1 day).

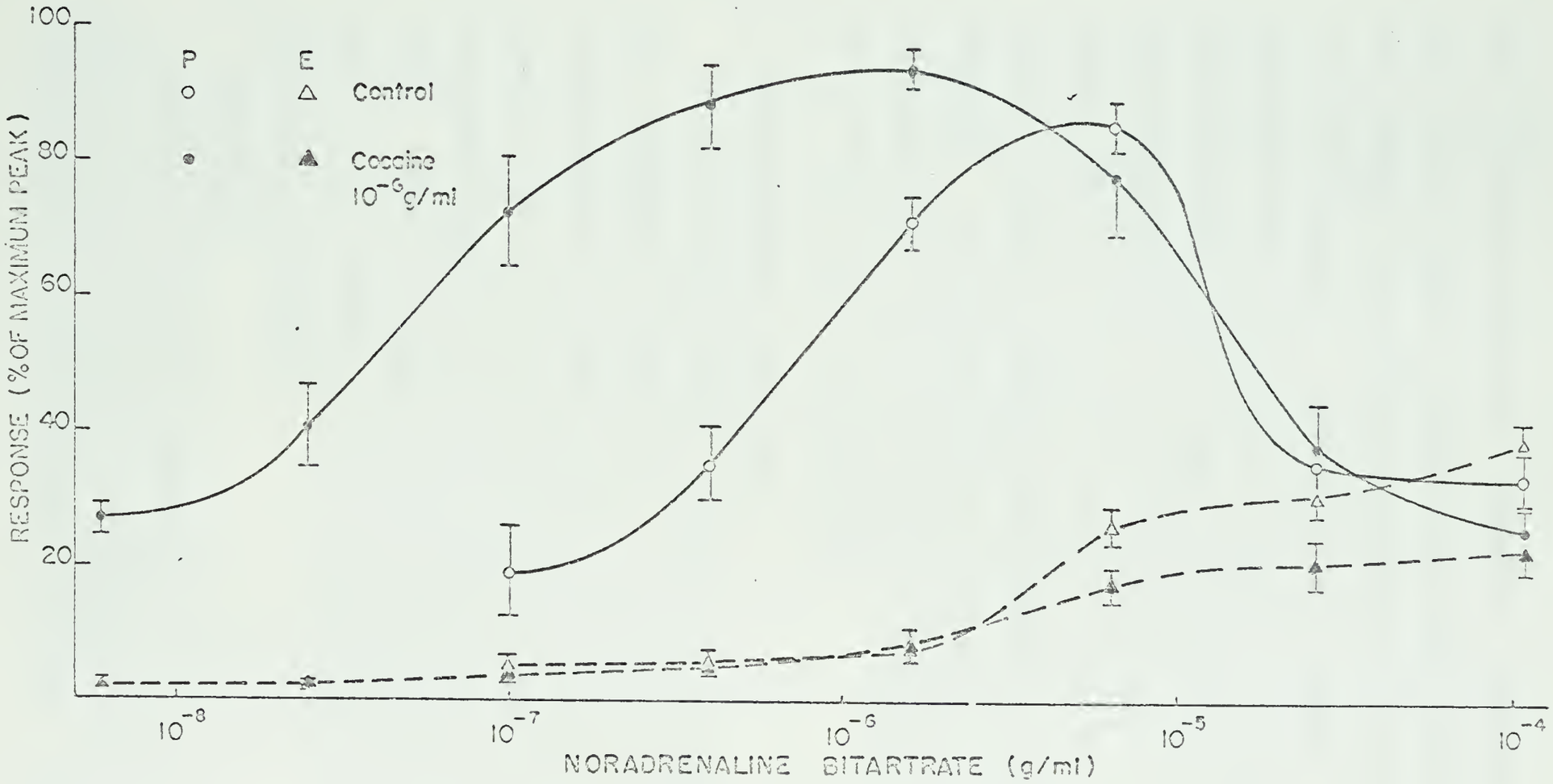


Figure 9. Cumulative Dose, Auxotonic Dose-Response Curves After Reserpine and Pheniprazine Pretreatment.

Points represent the means of values obtained using auxotonic levers and cumulative doses of noradrenaline. Animals were pretreated with reserpine (5 mg/Kg, I.P., 1 day) and pheniprazine (10 mg/Kg, I.P., 1 day).

alone, as shown in Figure 10. The shift of dose-response curves for peaks was not changed as cocaine still caused a leftward shift of 1 cycle, however the equilibria were depressed so severely that they were too small for analysis. These observations suggest that reserpine was responsible for the depression of equilibria in Figure 8. On comparison with control results (Figure 3), pretreatment with pheniprazine alone had little effect on the dose-response curves for peaks; the leftward shift due to cocaine was still 1 cycle and cocaine still caused an increase of maximum peaks although this effect was reduced (Figure 11). After pheniprazine pretreatment the equilibria were increased slightly, hence the equilibria depression seen in Figure 8 was not likely caused by pheniprazine.

B. REDUCED RECEPTOR CONCENTRATION

Prior to testing, maximal responses of the tissues were obtained with a supramaximal concentration of noradrenaline (10^{-4} g/ml). Then the tissues were subjected to phenoxybenzamine (5×10^{-9} g/ml) for 3 minutes so that part of the alpha receptor population was blocked and the maximum response to the above concentration of noradrenaline was reduced. Hence the maximum responses after phenoxybenzamine were limited by the efficiency of the receptors rather than by the physical limitations of the muscle.

a. Single Doses

Figure 12 shows typical responses of a tissue, with a limited population of alpha receptors, to supramaximal doses of noradrenaline in the absence of and in the presence of cocaine. After phenoxy-

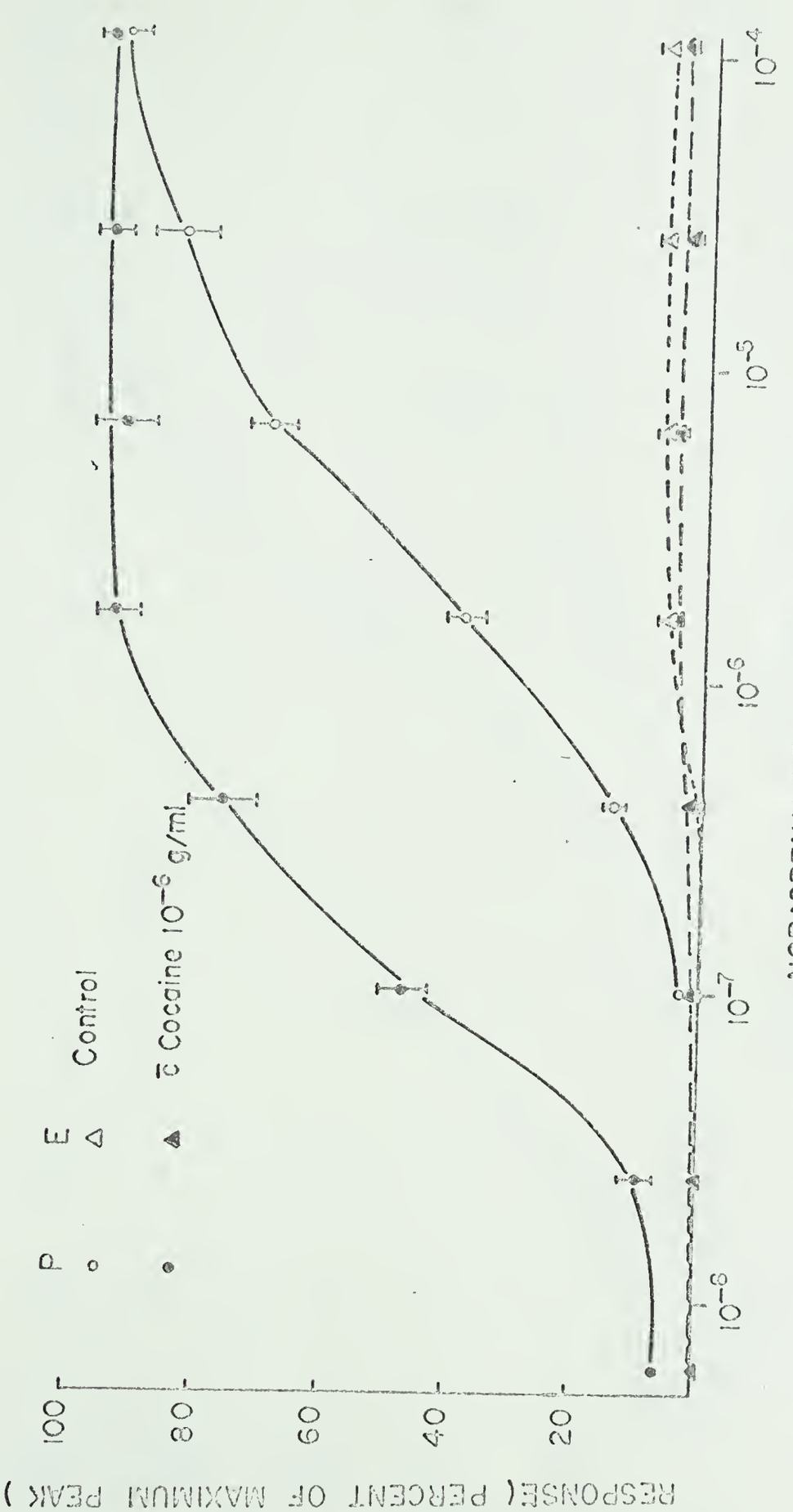


Figure 10. Single Dose, Isotonic Dose-Response Curves After Reserpine Pretreatment.

Points represent the means of values obtained using isotonic levers and single doses of noradrenaline. Animals were pretreated with reserpine (5 mg/Kg, I.P., 1 day).

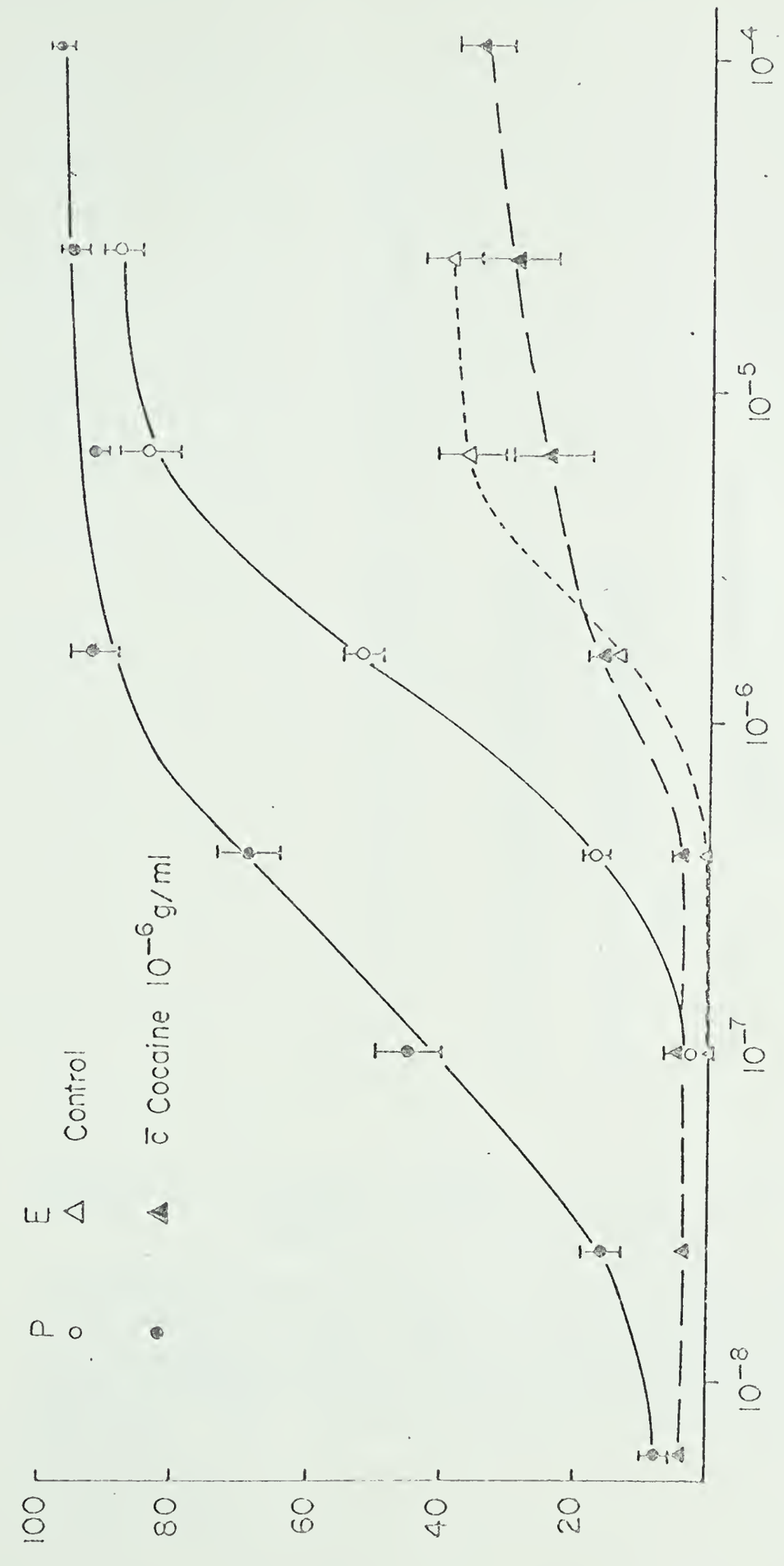


Figure 11. Single Dose, Isotonic Dose-Response Curves After Pheniprazine Pretreatment.

Points represent means of values obtained using isotonic levers and single dose administration of noradrenaline. Animals were pretreated with pheniprazine (10 mg/Kg, I.P., 1 day).

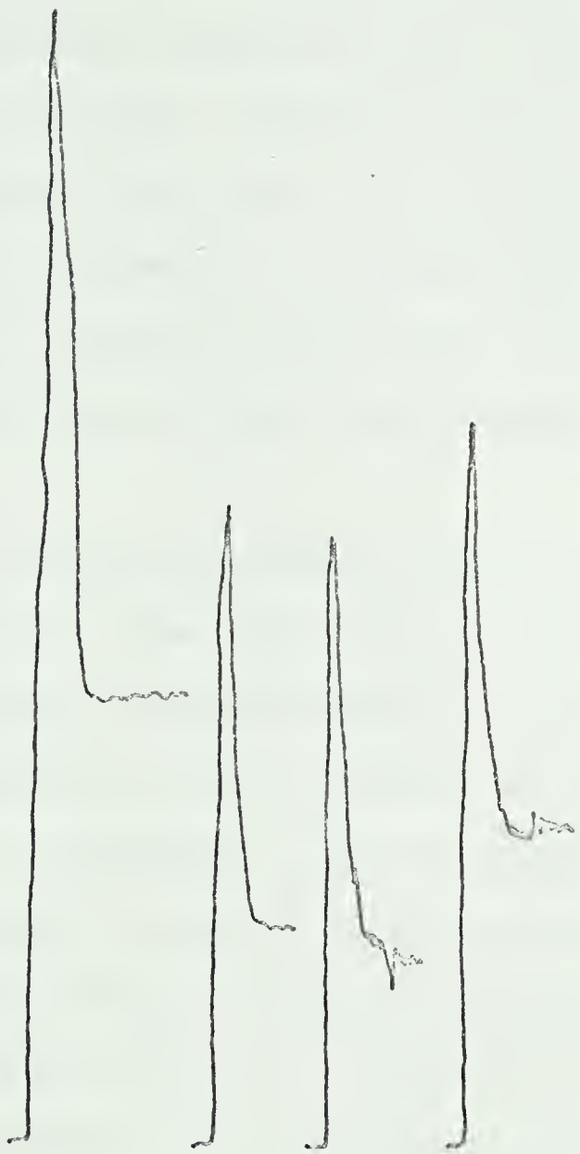


Figure 12. Tracings of Maximal Responses of Vas Deferens After Phenoxybenzamine Treatment.

Tracings represent responses to a supramaximal concentration of noradrenaline (10^{-4} g/ml) in the following order: control, control after partial blockade of alpha receptors with phenoxybenzamine (5×10^{-9} g/ml for 3 minutes), control after partial blockade and with cocaine (10^{-6} g/ml) after partial blockade.

1 cm = 5 min.

benzamine a supramaximal dose of noradrenaline could not elicit the maximum response of which the tissue was capable; however the presence of cocaine significantly increased the peak and equilibrium responses of these tissues (Figure 13). Since a supramaximal concentration of noradrenaline was used, it is assumed that all unblocked receptors were utilized, therefore the efficiency of the receptors or drug-receptor interactions must have been increased.

b. Cumulative Doses

The response of a tissue with limited receptors to a second large dose of noradrenaline added cumulatively is shown in Figure 14. The increased concentration of noradrenaline did not increase the magnitude of the equilibrium contraction (Figures 14 & 15), which shows that this dose is still supramaximal following the POB blockade. The fact that cocaine increased the equilibria after supramaximal concentrations of noradrenaline (Figure 15) supports the findings of the single dose experiments and confirms an increase of receptor efficiency. Although the standard errors of the means overlap, the increase was found to be significant ($P < 0.02$) on the basis of Student's t-test for paired data.

c. Methacholine Controls

Tissues were prepared and treated with phenoxybenzamine as above. The mean maximum response to a supramaximal concentration of methacholine (10^{-4} g/ml) after phenoxybenzamine was 52 $\pm$ 8.4% of the tissue maximum to noradrenaline (10^{-4} g/ml) before phenoxybenzamine. In the presence of cocaine the mean maximum response was 52 $\pm$ 9.0% of the

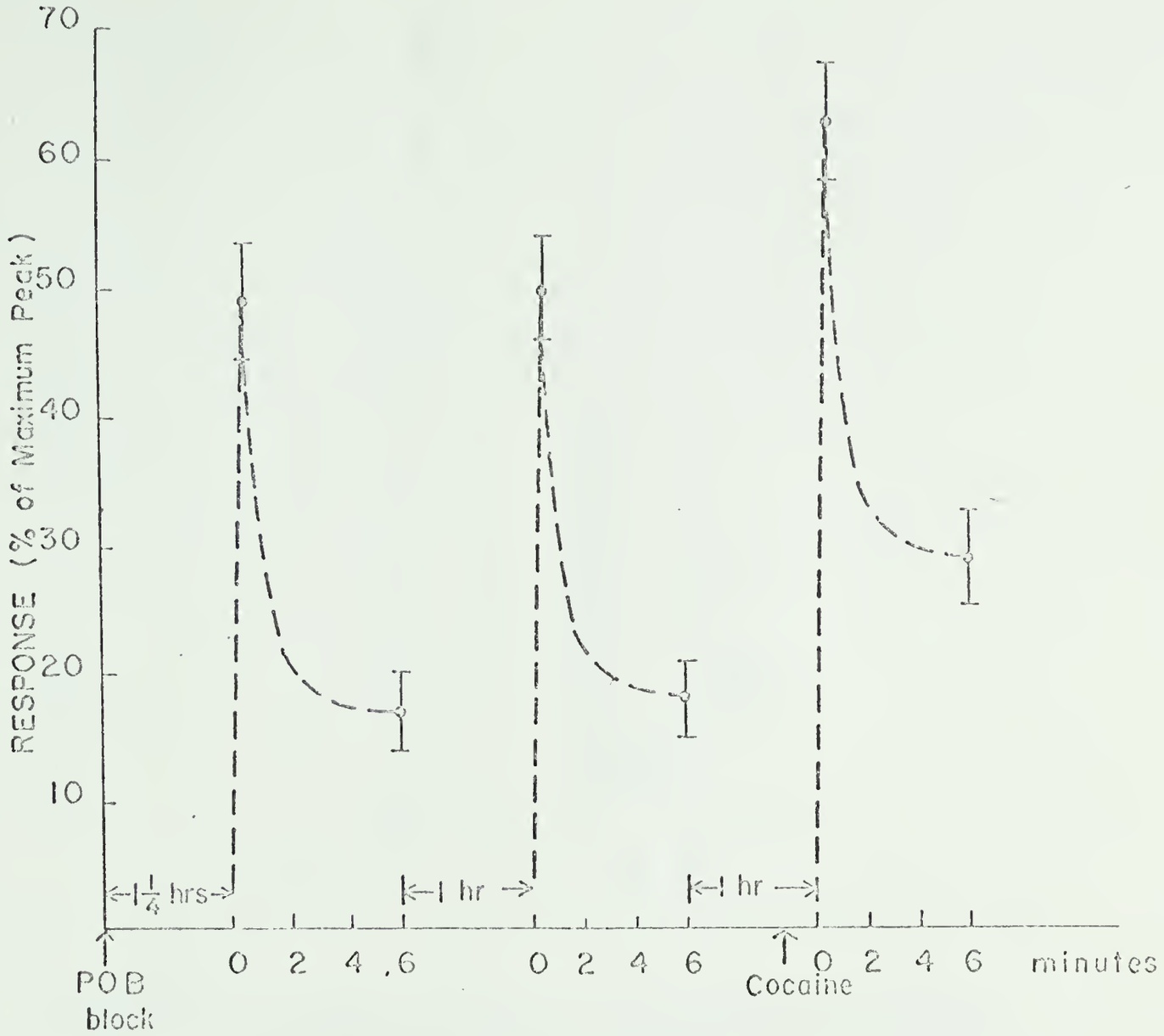


Figure 13. Maximal Responses After Phenoxybenzamine Treatment.

Figures represent responses to noradrenaline (10^{-4} g/ml) after treatment with phenoxybenzamine (5 X 10^{-9} g/ml for 3 minutes) in the following order: control, control and with cocaine. Points represent means of values obtained from tissues of 6 animals.

Figure 14. Tracings of Responses of Vas Deferens to Cocaine After Phenoxybenzamine Treatment and Supramaximal Concentrations of Noradrenaline.

First tracing represents control response to noradrenaline (10^{-4} g/ml).

Second tracing represents responses to noradrenaline (10^{-4} g/ml), noradrenaline (2 X 10^{-4} g/ml, first arrow), and cocaine (10^{-6} g/ml, second arrow) administered cumulatively after partial blockade of alpha receptors with phenoxybenzamine (5 X 10^{-9} g/ml for 3 minutes).

1 cm = 5 min.

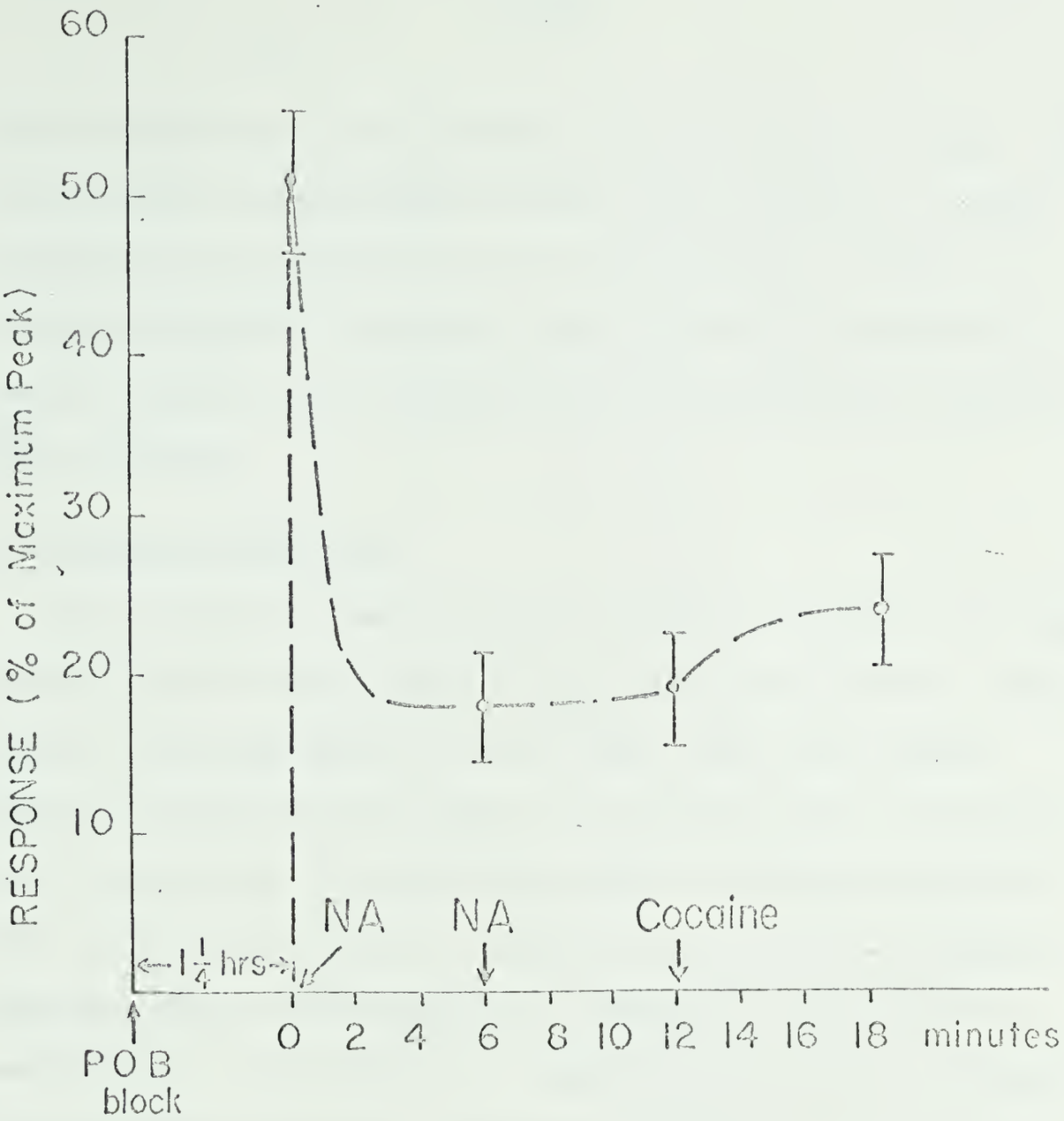


Figure 15. Response to Cocaine After Phenoxybenzamine Treatment and Supramaximal Concentrations of Noradrenaline.

Points represent means of values obtained from tissues of 6 animals. At 6 minutes the concentration of noradrenaline was increased from 10^{-4} g/ml to 2 X 10^{-4} g/ml and at 12 minutes cocain (10^{-6} g/ml) was added.

tissue maximum which is not different from that without cocaine. These results confirm the conclusions of Karr & Innes (1966) that cocaine supersensitivity is specific for the alpha adrenergic receptor; if cocaine had induced a nonspecific supersensitivity the methacholine response should have been increased as the tissue was still capable of more contraction.

C. PROTECTION EXPERIMENTS

These experiments were carried out to see if cocaine could either increase noradrenaline-receptor affinity or increase the rate of dissociation following reports by Karr & Innes (1966) that phenoxybenzamine blockade in the presence of a protecting dose of noradrenaline occurred faster if cocaine was present. A partial blockade of alpha receptors was effected by exposing one of a pair of tissues to phenoxybenzamine in the presence of a protecting concentration of noradrenaline. The contralateral tissue underwent the same treatment in the presence of cocaine. Subsequent to blockade both tissues were tested with a supramaximal concentration of noradrenaline; no significant difference was found between the responses of tissues blocked in the presence of and in the absence of cocaine (Figure 16). Therefore, cocaine did not alter the ability of noradrenaline to protect alpha receptors against phenoxybenzamine. Although only one concentration of noradrenaline was used, these results should be valid since the changes of the dose-response curve due to phenoxybenzamine are greatest at maximum concentrations of agonist. Phenoxybenzamine, in the rat vas deferens produces a reduction of slope, reduction of

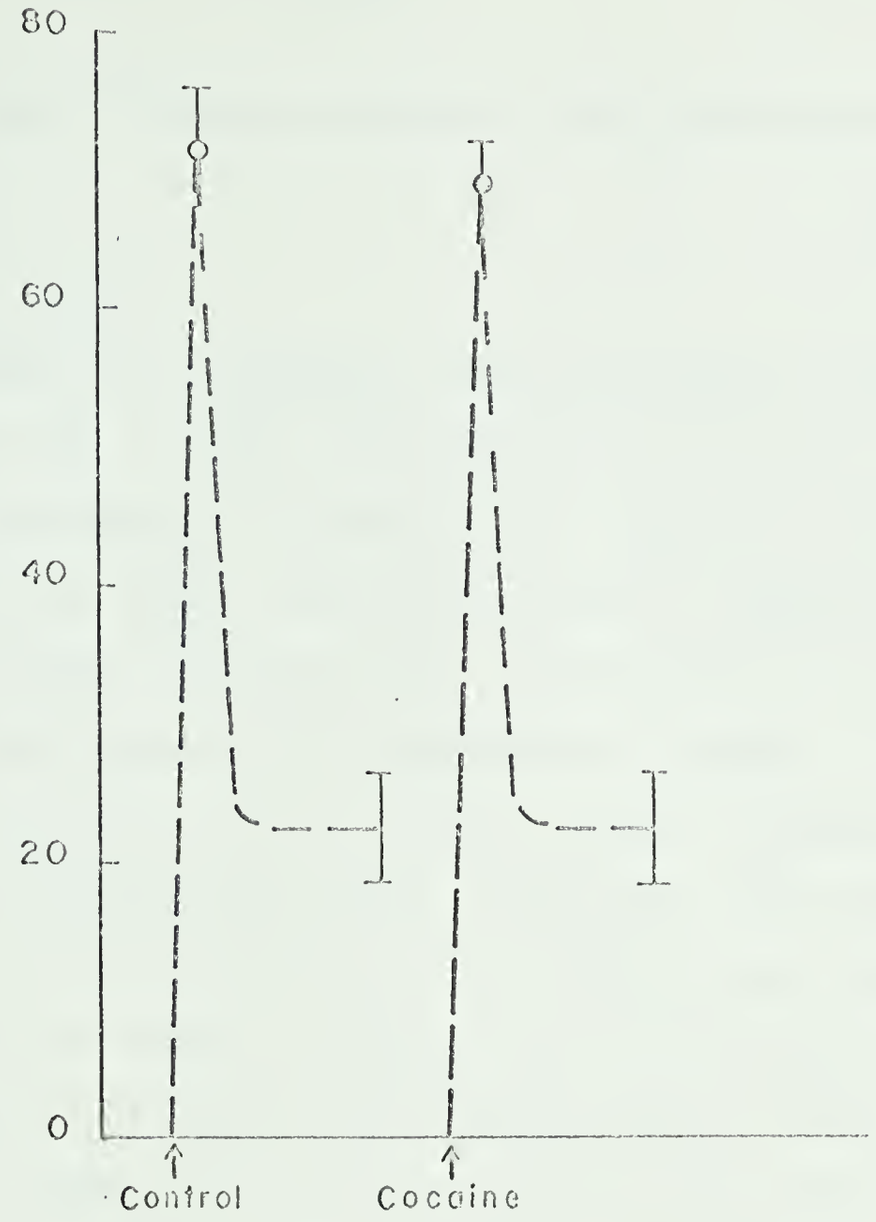


Figure 16. Effect of Cocaine on Phenoxybenzamine Blockade in Noradrenaline Protected Tissues.

First figure represents responses to noradrenaline (10^{-4} g/ml), after partial blockade by phenoxybenzamine (5 X 10^{-8} g/ml for 3 minutes) in the presence of a protecting concentration of noradrenaline (10^{-4} g/ml).

Second figure represents responses under the same conditions as first figure except cocaine (10^{-6} g/ml) was present during the partial blockade by phenoxybenzamine. Each point represents means of values from tissues of 12 rats.

maximum but little or no change in threshold of the dose-response curve to noradrenaline.

D. INHIBITION OF UPTAKE

Phenoxybenzamine can irreversibly inhibit the uptake of noradrenaline (Gillespie, 1965; Furchgott, 1966) therefore a reduction of sensitization by phenoxybenzamine treatment would be supportive evidence for the postulate that uptake inhibition by cocaine is responsible for at least part of cocaine supersensitivity. Sensitization was calculated as the ratio of the concentration of noradrenaline, required to produce a certain effect, before cocaine to that after cocaine. Readings made at a central point of the linear portion of the curves (45% of maximum response for control and 35% for phenoxybenzamine treated) showed that sensitization was reduced in tissues pretreated with phenoxybenzamine (Figure 17). Sensitization for phenoxybenzamine pretreated tissues was 10.1, while that for controls was 11.9; these values are significantly different with $P<0.005$. These observations indicate a role for uptake inhibition in the explanation of cocaine supersensitivity.

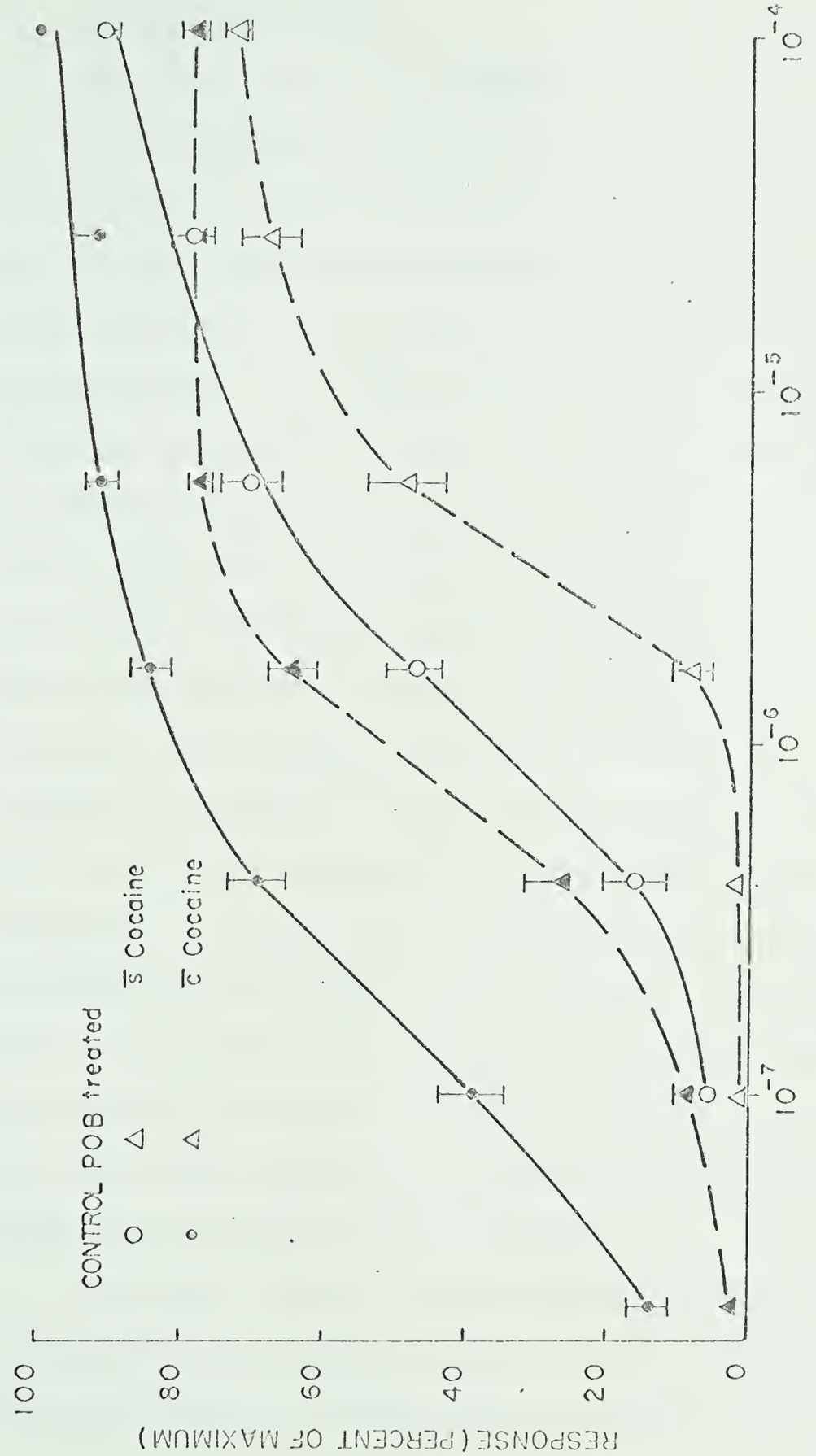


Figure 17. Effect of Phenoxybenzamine Treatment on Magnitude of Sensitization.

Points represent means of values obtained from tissues of 6 animals; peaks only are represented. Phenoxybenzamine treatment (broken lines) reduced the magnitude of sensitization by cocaine compared to untreated controls.

DISCUSSION

A. DOSE-RESPONSE CURVES

a. Normal Responses of Vas Deferens

The typical responses to single doses of noradrenaline (Figure 2) are difficult to explain in terms of the "occupation hypothesis" of drug-receptor excitation as discussed by Paton (1961). The rapid rise (peak) followed by fade to equilibrium might be explained by two types of smooth muscle; one with low threshold, fast action and fast accommodation and the other with higher threshold, slow action and gradual acquisition of steady state response. Another explanation might be developed on the basis of inhibitory receptors activated after excitatory receptors but inhibitory receptors do not exist in the smooth muscle of the rat vas deferens (Van Rossum, 1965; Vohra & Reiffenstein, 1967). Furchgott (1964) suggested that fade is caused by an "unspecific non-competitive antagonism" in the cholinergic system and this possibility should not be left unmentioned in the adrenergic system. However these responses are explained quite simply if they are interpreted with reference to Paton's (1961) "rate hypothesis" of drug-receptor excitation. The fast contraction can be explained by an initial rapid combination of drug molecules and receptors due to an abundance of free receptors and the steady state explained by a reduced combination rate limited by the number of free receptors at equilibrium.

The leftward shift of the dose-response curve to single doses of noradrenaline after cocaine (Figures 3 and 4) conforms to any of the theories. However, the slope would be expected to decrease on the basis

of "uptake theory" and increase on the basis of "alteration theory." By "uptake theory" the greatest potentiation should occur when inhibition of noradrenaline uptake is greatest. Since the concentration of cocaine was kept constant and since cocaine is a competitive inhibitor of uptake (Iversen, 1967) potentiation should be greatest at lowest concentrations of noradrenaline and least at the highest concentrations. Even if uptake of noradrenaline were completely blocked this would be true because the relative increase in local concentration of noradrenaline would be much greater at low doses than at high doses. However, it is unlikely that uptake was completely inhibited because Iversen (1967) found that concentrations of cocaine used here (10^{-6} g/ml) only inhibited uptake by 68% while 10^{-5} g/ml inhibited uptake by 95%. By "alteration theory", increased efficacy or intrinsic activity is postulated as the mode of action of supersensitivity. Therefore, if it were assumed that a constant proportion of receptors was activated by a constant concentration of noradrenaline then each response at that concentration would be expected to be increased proportionally. Hence the slope would necessarily be greater and potentiation greatest at high doses and least at low doses; this however was not the case.

Although neither of these theories by itself satisfactorily explains the pattern of potentiation found, a combination of the two would be suitable. The ratio of their importance would then be in proportion to their effect on the slope of the curve. This explanation is satisfactory for both peaks and equilibria regardless of whether interpretation is made on the basis of the "occupation" or

"rate hypothesis." If the "rate hypothesis" were used then an increase in the rate of dissociation, which would be equivalent to an increase of efficacy, should have been reflected by an increase of fade-ratio.

Since the values of fade-ratio were not changed by cocaine it is unlikely that cocaine altered the kinetics of noradrenaline-receptor interaction. If cocaine had increased the dissociation rate constant, the value for fade-ratio $(\frac{k_2}{k_1x + k_2})$ should have been increased. However an effective increase in agonist concentration (x) would decrease the fade-ratio and therefore mask an increase in k_2.

The responses of the vas deferens to cumulative doses (Figure 5) are nicely described by the "rate hypothesis." Since the response is, in some manner, proportional to the rate of association (A), where

$A = k_1x(1 - p)$

k_1 = association rate constant

x = drug concentration

p = proportion of receptors occupied

then the peak responses should decrease as the magnitude of p increases. The bell shaped curve for peaks is predicted by the "rate hypothesis." On the other hand, after a particular drug concentration has been present for a few minutes the equation for A at equilibrium conditions explains the dose-response curves for steady state contractions.

$$A = \frac{k_2x}{x + \frac{k_2}{k_1}}$$

k_2 = dissociation rate constant

Here A does not decrease with an increase in drug concentration and no autoinhibition is seen.

The same shift of curves for peaks found with cumulative dose curves as with single dose curves is explained by any of the theories. However, the increase of maximal peak is explained by "uptake theory" only if the "rate hypothesis" for excitation is used because the "occupation hypothesis" does not allow for an increase of the maximum response due to increase of the concentration of agonist. On the other hand if "alteration theory" is used then it is possible to explain the increase of maximum response by either an increase of efficacy (Stephenson, 1956) or an increase in rate of dissociation. Furthermore, the small shift or lack of shift of the declining portions of these curves (Figures 6 & 7) supports both "uptake" and "alteration theories." According to "uptake theory" the shift of the curves at the concentrations of noradrenaline found in this region should be small because cocaine, at these noradrenaline concentrations, cannot significantly increase the concentration of agonist near the receptors (Iversen, 1965). No leftward shift of the declining portion of the dose-response curve was predicted on the basis of "alteration theory" because if cocaine caused an increase of intrinsic activity there would be no reason for responses, at any concentration of agonist, to be reduced. If analysis is based on the "rate hypothesis" and k_2 is assumed to be increased, no large difference in peaks at this point in the dose-response curve would be predicted because of the large proportion of receptors occupied due to the previous high concentration

of noradrenaline. The reduced equilibrium responses in the presence of cocaine support neither "uptake" nor "alteration theory." The explanation for this depression may lie in a cocaine induced desensitization to noradrenaline which manifests itself after long exposure. If this were correct then the program of drug administration was inappropriate for the analysis of equilibria.

b. Saturated Stores

Since granular stores and intracellular catabolism of noradrenaline were eliminated (Van Orden et al, 1967), it is assumed that the storage depots for noradrenaline were saturable. The storage depots, now limited to the cytoplasm, would only be able to accumulate noradrenaline to the point where uptake would be matched by loss due to diffusion back across the cell membrane. Therefore, if the storage depots were saturated after the first few doses of noradrenaline, no supersensitivity due to blockade of uptake by cocaine would have been predicted on the basis of "uptake theory." If the nerves were no longer capable of net uptake of noradrenaline then cocaine inhibition of uptake could not increase the local concentration at the receptor site. The fact that the peaks on the cumulative dose-response curves (Figure 9) were potentiated fails to support "uptake theory", especially if responses are interpreted according to the "occupation hypothesis." On the basis of the "rate hypothesis" the possibility exists that the dissociation rate constant (k_2) increases, hence the equilibrium would also be expected to increase according to the equation

$$A = \frac{k_2 x}{x + \frac{k_2}{k_1}}$$

However the effect of pretreatment was to depress the magnitude of the equilibria so severely that these could not be analysed. Unfortunately, this also eliminated the opportunity to analyse fade (Paton, 1961) on these curves.

The results obtained for equilibria support none of the theories proposed in explanation of cocaine supersensitivity; the depression of equilibria in both single dose, isotonic and cumulative dose, auxotonic dose-response curves (Figures 8 & 9) was sufficient that meaningful changes were not found after cocaine. There may have been a different factor responsible for the depression of equilibria; this led to the testing of pheniprazine or reserpine pretreatment individually.

c. Effects of Reserpine or Pheniprazine Pretreatment

Pretreatment with pheniprazine alone did not lead to any depression of equilibria and curves (Figure 11) obtained from this experiment were similar to those obtained from tissues of untreated animals. Any changes found were compatible with the monoamine oxidase inhibiting properties of pheniprazine.

On the other hand reserpine pretreatment while not affecting peaks depressed the equilibria (Figure 10). The reason for this is not immediately clear but may involve changes in tissue ion content. It is known that alterations in the ion concentrations of extracellular fluid and the muscle itself lead to distortions in responses (Davson,

1964). Carrier et al (1967) showed that reserpine produced large changes in the sodium and potassium content of both dog and rat vasculature. Carrier and Shibata (1967) found that reserpine (5 mg/kg, I.P., 24 hours) reduced rat aortic calcium by 20%. These ionic alterations may be the cause of the inability of vas deferens to maintain the equilibrium contraction after pretreatment with reserpine. From these findings, it is apparent that the use of reserpine is not suitable for this particular experimental design.

B. REDUCED RECEPTOR CONCENTRATION

a. Single Doses

Figures 12 and 13 show the increased responses, after cocaine, from tissues whose alpha receptors have been partially blocked by phenoxybenzamine. Since these tissues were limited in their responses to noradrenaline only by the receptor-excitation system rather than by mechanical limits of the tissue, any increase in responses in the presence of cocaine must be a receptor-excitation phenomenon. The fact that a supramaximal concentration of noradrenaline (10^{-4} g/ml) was used rules out the possibility that the potentiated responses were caused by increased local concentrations of noradrenaline or an increase of affinity. Therefore the potentiating effect of cocaine must have been due to a direct action on the effector cells themselves.

The phenomenon seen must have been caused by production of amplified stimulus by the same number of receptors, when cocaine was present. By the "occupation hypothesis", "intrinsic activity" (Ariens, 1964) or "efficacy" (Stephenson, 1956) must have been increased.

Greenberg & Innes (1968) have suggested that noradrenaline "utilizes less extracellular Ca^{++} for contraction in the presence of cocaine. This may be due to cocaine releasing a bound intracellular store." Daniel & Wolowyk (1966) also suggested that cocaine induces labilization of Ca^{++} but they speculated that this occurred in the cell membrane, at least in the rat uterus. Therefore it might be possible that cocaine primes a labile Ca^{++} pool (utilized specifically by the alpha receptor) from which Ca^{++} can be released by alpha receptor stimulation. Also on the basis of the "occupation hypothesis" the affinity of the receptor for noradrenaline may have been increased, however this possibility is ruled out by the use of supramaximal concentrations. Tuttle (1968) suggested that "Ca^{++} facilitates the combination of noradrenaline with the adrenergic receptor." However, since these experiments indicate otherwise (i.e. no affinity change is likely) it may be that Ca^{++} increases the rate of turnover of drug-receptor combinations.

An increased rate of turnover would necessarily involve an increase in the rate of dissociation. In this case the peaks would be potentiated because the period of rapid combination of drug and receptor would be increased due to the higher rate of dissociation. Furthermore, the increase of maximal equilibrium conforms to an increase of k_2. For maximal equilibrium Paton's (1961) "rate hypothesis" equation can be expressed as

$$y_{max} = \phi k_2$$

where y = equilibrium contraction

ϕ = constant relating stimulus to response (including excitation-

contraction coupling).

Clearly then an increase in k_2 would produce an increase in y_{max}. Although an increase in ϕ would also produce an increase in y_{max}, this possibility appears to be excluded by these tests done using methacholine. The possibility that cocaine induces an increase in k_2 has been tested in the protection experiments.

b. Cumulative Doses

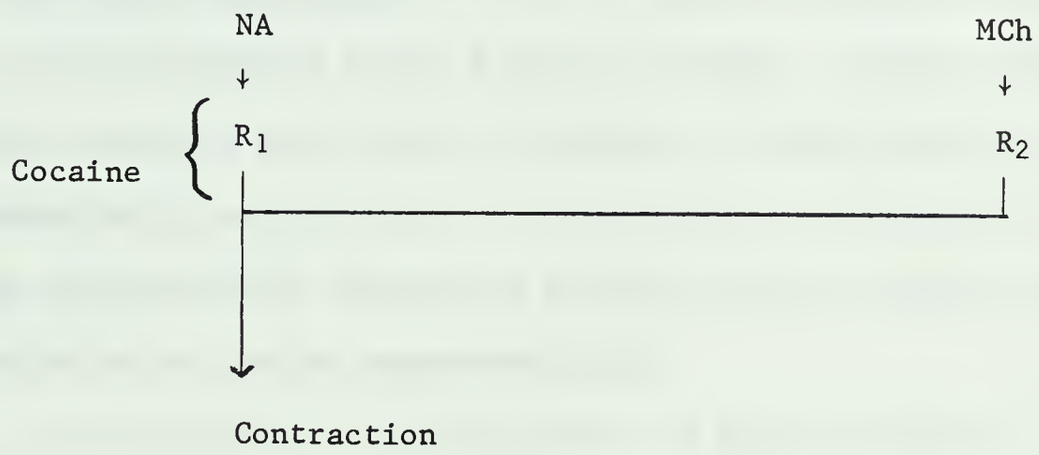
The main purpose of this experiment was to ensure that the concentration of noradrenaline used (10^{-4} g/ml) was still maximal after phenoxybenzamine treatment. In normal tissues it was quite possible that maximal responses were attained at lower concentrations of noradrenaline than those required for maximal stimulus. Therefore the potentiality that the concentration of noradrenaline used was not supramaximal for stimulus required testing. Since no further increase in response was observed after doubling the concentration of noradrenaline (Figures 14 and 15), 10^{-4} g/ml was considered sufficient to be supramaximal in these experiments.

After two doses of noradrenaline, the increased contraction which resulted from the cumulative addition of cocaine lends further support to the inferences made on the basis of single dose experiments. The maximal equilibrium attained by cumulative doses (Figure 15) was less than that attained by single doses (Figure 13). This can be attributed to either protection of receptors by noradrenaline, which may have interfered with the combination of cocaine and receptor (Karr & Innes, 1966), or inability of the tissue to respond further as a result

of the prolonged (12 minute) exposure to high concentrations of noradrenaline (1 to 2 X 10^{-4} g/ml).

c. Methacholine Controls

This experiment was performed to ascertain the specificity of cocaine supersensitivity. Since methacholine responses were not found to be potentiated by cocaine the action of cocaine must be prior to the junction of adrenergic and cholinergic pathways on excitation-contraction coupling.



C. PROTECTION EXPERIMENTS

The purpose of these experiments was to see if cocaine altered the kinetics of drug-receptor interaction. It was suggested earlier in this discussion that cocaine could produce supersensitivity by increasing the rate of dissociation (k_2), according to Paton's (1961) "rate hypothesis." If this were the case a more complete blockade, by phenoxybenzamine, of alpha receptors would have been expected. If k_2 was increased, the number of free receptors would have been greater at any given instant; therefore, phenoxybenzamine would have had a better

opportunity to combine with the receptors. However, no increase in blockade by phenoxybenzamine was found (Figure 16), therefore, cocaine does not appear to potentiate by increasing the rate of drug-receptor dissociation. On the other hand, it is possible that cocaine altered the kinetics of phenoxybenzamine and alpha receptor combination in a manner that compensated for an increase in the rate of dissociation of the noradrenaline-receptor complex.

On the other hand, if cocaine produced supersensitivity by increasing noradrenaline-receptor affinity, blockade of alpha receptors by phenoxybenzamine should have been reduced. Greater affinity should have increased the portion of receptors protected against phenoxybenzamine by noradrenaline. Yet no increase of receptor protection was observed hence increase of affinity does not appear to be the mechanism of cocaine supersensitivity.

One possibility for the failure to find any change in protection due to cocaine is that the duration of exposure to phenoxybenzamine may have been too great. Perhaps a difference would have been found had a shorter duration been used because with increased duration any changes in noradrenaline-receptor affinity or dissociation would become less noticeable. Since the rate of phenoxybenzamine-receptor combination is likely greatest just after the tissue has been exposed to phenoxybenzamine, any change in rate of blockade or protection by cocaine would probably be most evident at this time.

On the basis of these results, cocaine does not produce supersensitivity by increasing affinity and probably not by increasing the

rate of dissociation. It may, however, act at or very near the receptor to produce its effect. Experiments using methacholine as agonist suggest that cocaine acts close to the alpha receptor. Furthermore, Vohra (1968) presented evidence to support a receptor or parareceptor site of cocaine action. He showed that cocaine, in concentrations greater than used here, could produce contractions in vasa deferentia taken from both normal and reserpinized rats. In addition, these responses to cocaine could be blocked by alpha adrenergic blocking agents.

D. INHIBITION OF UPTAKE

As it was shown that cocaine had a direct action on the effector cells to cause potentiation, the status of uptake of noradrenaline in causing potentiation in this preparation was uncertain. However, this experiment confirms the previous hypothesis that both "alteration theory" and "uptake theory" are applicable. Cocaine produced a significantly reduced supersensitivity in tissues treated with phenoxybenzamine to block uptake sites compared to untreated controls (Figure 17). Unfortunately, uptake studies were not carried out, therefore, only limited significance can be placed on these experiments. Furthermore, phenoxybenzamine may have blocked the postsynaptic site of cocaine activity, rather than, or in addition to inhibition of uptake, to reduce the magnitude of sensitization.

If both theories are useful and indeed necessary in the explanation of cocaine supersensitivity then their relative importance is pertinent. On the basis of experiments performed by de la Lande & Waterson (1967) on the isolated artery of the rabbit ear, the inhibition

of uptake may be 5 times as effective as the direct effect of cocaine on the muscle. Extrapolating from this work then, uptake imhibition may be most important in highly innervated tissues and demonstration of a direct component of sensitization in these tissues possible only under certain conditions. This may explain the wide application of "uptake theory" in describing this phenomenon and also the more limited use but indispensability of "alteration theory."

CONCLUSIONS

a. Responses of the rat vas deferens to noradrenaline are described most simply by the "rate hypothesis" (Paton, 1961) of receptor activation. Characteristically, responses consist of a rapid contraction (peak) followed by a relaxation to a steady state contraction (equilibrium). The more powerful peak contraction matches the high rate of drug-receptor combination expected immediately after exposure of the tissue to agonist; the less powerful equilibrium contraction matches the reduced rate of drug-receptor combination expected after a certain proportion of the receptors have been occupied. In addition, the peaks at high concentrations were reduced when noradrenaline was administered on a cumulative schedule; this would have been expected since the rate of drug-receptor combination should be reduced by the previous concentration of noradrenaline.

b. The presence of cocaine (10^{-6} g/ml) in the bath causes a 10 fold sensitization of responses to noradrenaline (shift of dose-response curve to lower concentrations of noradrenaline) except in the case of high concentrations of noradrenaline administered on a cumulative basis. Since the slopes of the curves obtained in the presence of cocaine were not changed from the controls, it is apparent that both inhibition of uptake and a direct effect on the muscle itself are involved in cocaine supersensitivity.

c. After noradrenaline storage depots were made saturable (by destruction of granular function with reserpine and inhibition of monoamine oxidase by pheniprazine) dose-response curves for peaks were

still shifted by cocaine with both single dose and cumulative dose administration of noradrenaline. On the assumption that the storage depots were saturated after the first few doses of noradrenaline when cumulative administration was used, cocaine should not have produced supersensitivity to noradrenaline by inhibition of uptake since no net uptake would be possible under these conditions. These results indicate that inhibition of uptake is not an adequate explanation of cocaine supersensitivity. The equilibria obtained from these tissues was depressed sufficiently that they could not be analysed. Both reserpine and pheniprazine pretreatment alone were tested to see if either was responsible for the reduction of steady state responses. While pheniprazine had little effect on these responses, reserpine caused a severe reduction; therefore, reserpine was not a suitable drug for the destruction of granular function in this tissue.

d. After tissues had been treated with phenoxybenzamine so that their maximal responses were limited by the properties of the receptors and not by contractile confines, cocaine induced an increase in the maximal responses which these tissues could produce. The possibility that inhibition of uptake caused this potentiation is ruled out by the supramaximal concentrations of noradrenaline used; therefore, it is concluded that cocaine acts directly on the muscle to produce supersensitivity. Experiments performed on these tissues using cumulative administration of drugs both confirm a direct action of cocaine and validate the use of 10^{-4} g/ml (noradrenaline) as a supramaximal dose.

e. After some of the uptake sites for noradrenaline were blocked

with phenoxybenzamine, the magnitude of cocaine supersensitivity (shift of dose-response curve) was reduced. Since this observation would have been predicted on the basis of "uptake theory", inhibition of uptake probably plays a role in cocaine supersensitivity in the rat vas deferens.

f. If cocaine affected the affinity or dissociation of the noradrenaline-receptor combination it should have altered the proportion of receptors occupied by a high concentration of noradrenaline. Therefore, the protection afforded by a protecting concentration of noradrenaline (10^{-4} g/ml) against phenoxybenzamine should have been changed by the presence of cocaine. However, cocaine induced no change in noradrenaline protection of alpha receptors, therefore it appeared (within the limitations discussed) that neither affinity nor dissociation was affected.

g. Since cocaine

i. did not change the slopes of dose-response curves

ii. still produced supersensitivity after noradrenaline storage depots were made saturable

iii. caused potentiation of maximal responses in tissues with limited receptors

iv. caused less supersensitivity in tissues with some of their uptake sites blocked by phenoxybenzamine and

v. did not appear to alter kinetics,

it is concluded that both inhibition of uptake and an increase of efficacy are required to explain cocaine supersensitivity in the rat vas deferens.

BIBLIOGRAPHY

ANDERSEN, N. B. & GRAVENSTEIN, J.C. (1965). Effects of local anesthetics on sodium and potassium in human red cells. J. Pharmac. exp. Ther., 147, 40-47.

ARIENS, E. J., SIMONIS, A. M. & VAN ROSSUM, J. M. (1964). Drug-receptor interaction: Interaction of one or more drugs with one receptor system. Molecular Pharmacology, ed. Ariens, E. J. pp. 137-139. New York: Academic Press.

AXELROD, J. (1957). O-Methylation of epinephrine and other catechols in vitro and in vivo. Science, N.Y. 126, 400.

AXELROD, J. (1966). Methylation reactions in the formation and metabolism of catecholamines and other biogenic amines. Pharmac. Rev., 18, 95-113.

BARNETT, A., GREENHOUSE, D. D. & TABER, R. I. (1968). A new type of drug enhancement: Increased maximum response to cumulative noradrenaline in the isolated rat vas deferens. Br. J. Pharmac. Chemother., 33, 171-176.

BEVAN, J. A. & VERITY, M. A. (1967). Sympathetic nerve-free vascular muscle. J. Pharmac. exp. Ther., 157, 117-124.

BLASCHKO, H. (1952). Amino oxidase and amine metabolism. Pharmac. Rev., 4, 415-458.

BURN, J. H. (1932). The action of tyramine and ephedrine. J. Pharmac. exp. Ther., 46, 75-95.

BURN, J. H. (1952). The enzyme at sympathetic nerve endings. Br. med. J., 1, 784-787.

BURN, J. H. & ROBINSON, J. (1952). Effect of denervation on amine oxidase in structures innervated by the sympathetic. Br. J. Pharmac. Chemother., 7, 304-318.

CARRIER, J., Jr., DOUGLAS, B. H., GARRETT, L., & WHITTINGTON, P. J. (1967). The effect of reserpine on vascular tissue sodium and potassium content. J. Pharmac. exp. Ther., 158, 494-503.

CARRIER, O., Jr., & SHIBATA, S. (1967). A possible role for tissue calcium in reserpine supersensitivity. J. Pharmac. exp. Ther., 155, 42-49.

CLARK, A. J. (1937). Handbuch Der Experimentellen Pharmakologie, Ergänzungswerk IV, p.188. Berlin: Springer.

CROUT, R. J., McANELLY, S. J. & TATUM, E. (1967). Mechanism of action of cocaine. Fedn. Proc., 26, 569.

DANIEL, E. E. & WOLOWYK, M. (1966). The contractile response of the uterus to cocaine. Can. J. Physiol. Pharmac., 44, 721-730.

DAVIDSON, W. J. & INNES, I. R. (1968). Relation of catecholamine uptake to supersensitivity due to cocaine. Can. Fed. Biol. Soc. Proc., 11, 126-127.

DAVSON, H. (1964). A Textbook of General Physiology, 3rd edn., p. 914-936. Boston: Little, Brown and Company.

DE LA LANDE, I. S., FREWIN, D., WATERSON, J., & CANELL, V. (1967) Factors influencing supersensitivity to noradrenaline in the isolated perfused artery; comparative effects of cocaine, denervation and serotonin. Circulation Res., 20 & 21, III, 177-181.

DE LA LANDE, I. S. & WATERSON, J. G. (1967). Site of action of cocaine on the perfused artery. Nature, Lond. 214, 313-314.

DRASKOCZY, P. R. (1967). The uptake of l-, and d-norepinephrine (ℓ-NE, d-NE) by the isolated perfused rabbit heart. Fedn. Proc., 26, 569.

FARRANT, J. (1963). Interactions between cocaine, tyramine and noradrenaline at the noradrenaline store. Br. J. Pharmac. Chemother., 20, 540-549.

FISHER, R. A. & YATES, F. (1963). Statistical Tables for Biological, Agricultural and Medical Research, 6th edn., p.44. Edinburgh: Oliver and Boyd.

FLECKENSTEIN, A. & BASS, H. (1953). Zum mechanismus der wirkungsuerstarkung und wirkungsabschwachung sympathomimetischer amine durch cocain und andere pharmaka. Arch. exp. Path. Pharmak., 220, 143-156. Cited by Furchgott, R. F. (1955). Pharmac. Rev., 7, 183-265.

FLECKENSTEIN, A. & BURN, J. H. (1953). The effect of denervation on the action of sympathomimetic amines on the nictitating membrane. Br. J. Pharmac. Chemother., 8: 69-78.

FROHLICH, A. & LOEWI, O. (1910). Uber eine steigerung der adrenalinempfindlichkeit durch cocain. Arch. exp. Path. Pharmak., 62, 159-169.

FURCHGOTT, R. F. (1955). The pharmacology of vascular smooth muscle. Pharmac. Rev., 7, 183-265.

FURCHGOTT, R. F., KIRPEKAR, S. M., RIEKER, M. & SCHWAB, A. (1963). Actions and interactions of norepinephrine, tyramine and cocaine on aortic strips of rabbit and left atria of guinea pig and cat. J. Pharmac. exp. Ther., 142, 39-58.

FURCHGOTT, R. F. (1964). Receptor mechanisms. Ann. Rev. Pharmac., 4, 21-50.

FURCHGOTT, R. F. (1966). The use of β-haloalkylamines in the differentiation of receptors and in the determination of dissociation constants of receptor-agonist complexes. Advances in Drug Research, vol. 3, ed. Harper, N. J. & Simmonds, A. B., pp.31-35. London: Academic Press.

GADDUM, J. H. & KWIATKOWSKI, H. (1938). The action of ephedrine. J. Physiol., 94, 87-100.

GILLESPIE, J. S. & KIRPEKAR, S. M. (1965). The inactivation of infused noradrenaline by the cat spleen. J. Physiol., 176, 205-227.

GREENBERG, R. & INNES, I. R. (1968). The role of calcium in cocaine supersensitivity to norepinephrine. Fedn. Proc., 27, 599.

HARDMAN, J. G, Mayer, S. E., & CLARK, B. (1965). Cocaine potentiation of the cardiac inotropic and phosphorylase responses to catecholamines as related to the uptake of H^3-catecholamines. J. Pharmac. exp. Ther., 150, 341-348.

IVERSEN, L. L. (1965). The uptake of catecholamines at high perfusion concentrations in the isolated rat heart: a novel catecholamine uptake process. Br. J. Pharmac. Chemother., 25, 18-33.

IVERSEN, L. L. (1967). The uptake and storage of noradrenaline in sympathetic nerves. London: Cambridge University Press.

JANG, C-S. (1940). Interaction of sympathomimetic substances on adrenergic transmission. J. Pharmac. exp. Ther., 70, 347-361.

KARR, G. W. & INNES, I. R. (1966). Protection against supersensitivity induced by cocaine in smooth muscle. Can. Fed. Biol. Soc. Proc., 9, 38-39.

KARR, G. W. & INNES, I. R. (1967). The role of receptor changes in supersensitivity to catecholamines. Can. Fed. Biol. Soc. Proc., 10, 58-59.

LESZKOVSKY, C. P. & TARDOS, L. (1968). Potentiation by cocaine and 3, 3-di(p-amino phenyl)-propylamine (TK174) of the effect of isoprenaline and noradrenaline on isolated strips of cat spleen. J. Pharm. Pharmac., 20, 377-380.

MacMILLAN, W. H. (1959). A hypothesis concerning the effect of cocaine on the action of sympathomimetic amines. Br. J. Pharmac. Chemother., 14, 385-391.

MAXWELL, R. A. (1965). Concerning the mechanism of action of methylphenidate on the responses of rabbit vascular tissue to norepinephrine. J. Pharmac. exp. Ther., 147, 289-297.

MAXWELL, R. A., SYLWESTROWICZ, H. D., HOLLAND, R., SCHNEIDER, F., & DANIEL, A. J. (1961). Some actions of methylphenidate on the vascular system, arterial tissue and the nictitating membrane. J. Pharmac. exp. Ther., 131, 355-365.

MAXWELL, R. A., Wastila, W. B., & ECKHARDT, S. B. (1966). Some factors determining the response of rabbit aortic strips to dl-norepinephrine-7-H^3 hydrochloride and the influence of cocaine, guanethidine and methylphenidate on these factors. J. Pharmac. exp. Ther., 151, 253-261.

Merck Index of Chemicals and Drugs (Sixth Edition). Merck & Co. Inc., Rohway, N.Y., U.S.A. (1952).

MUSCHOLL, E. (1961) Effect of cocaine and related drugs on the uptake of noradrenaline by heart and spleen. Br. J. Pharmac. Chemother., 16, 352-359.

NICKERSON, M., HENRY, J. W. & NOMAGUCHI, G. M. (1953). Blockade of responses to epinephrine and norepinephrine by dibenamine congeners. J. Pharmac. exp. Ther., 107, 300-309.

PATON, W. D. M. (1957). A pendulum auxotonic lever. J. Physiol., 137, 35P-36P.

PATON, W. D. M. (1961). A theory of drug action based on the rate of drug-receptor combination. Proc. Roy. Soc. London B, 154, 21-69.

PHILPOT, F. J. (1940). The inhibition of adrenaline oxidation by local anesthetics. J. Physiol., 97, 301-307.

REIFFENSTEIN, R. J. (1968). Effects of cocaine on the rate of contraction to noradrenaline in the cat spleen strip: Mode of action of cocaine. Br. J. Pharmac. Chemother., 32, 591-597.

STEPHENSON, R. P. (1956). A modification of receptor theory. Br. J. Pharmac. Chemother., 11, 379-393.

THOENEN, H., HUERLIMANN, A. & HAEFELY, W. (1964). Mode of action of imipramine and 5-(3^1-methyl-aminopropyliden)-dibenzo[a,e] cyclo-hepta[1,3,5]trien hydrochloride (RO4-604), a new antidepressant drug, on peripheral adrenergic mechanisms. J. Pharmac. exp. Ther., 144, 405-414.

TRENDELENBURG, U. (1959). The supersensitivity caused by cocaine. J. Pharmac. exp. Ther., 125, 55-65.

TRENDELENBURG, U. (1963). Supersensitivity and subsensitivity to sympathomimetic amines. Pharmac. Rev., 15, 225-276.

TRENDELENBURG, U. (1965). Supersensitivity by cocaine to destro-rotatory isomers of norepinephrine and epinephrine. J. Pharmac. exp. Ther., 148, 329-338.

TRENDELENBURG, U. (1966). Mechanisms of supersensitivity and subsensitivity to sympathomimetic amines. Pharmac. Rev., 18, 629-640.

TRENDELENBURG, U., MUSKUS, A., FLEMING, W. W. & GOMEZ ALONSO DE LA SIERRA, B. (1962a). Modification by reserpine of the action of sympathomimetic amines in spinal cats; a classification of sympathomimetic amines. J. Pharmac. exp. Ther., 138, 170-180.

TRENDELENBURG, U., MUSKUS, A., FLEMING, W. W. & GOMEZ ALONSO DE LA SIERRA, B. (1962b). Effect of cocaine, denervation and decentralization on the response of the nictitating membrane to various sympathomimetic amines. J. Pharmac. exp. Ther., 138, 181-193.

TUTTLE, R. R. (1968). Effect of calcium on combination of norepine-phrine with the alpha adrenergic receptor. Fedn. Proc., 27, 599.

VAN ORDEN, L. W., III, BLOOM, F. E., BARRNETT, R. J., & GIARMAN, N. J. (1967). Histochemical and functional relationships of catecholamines in adrenergic nerve endings. I. Participation of granular vesicles. J. Pharmac. exp. Ther., 154, 185-199.

VAN ROSSUM, J. M. (1965). Different types of sympathomimetic α-receptors. J. Pharm. Pharmac., 17, 202-216.

VOHRA, M. M. (1968). Response of rat and guinea pig vas deferens to cocaine and tetracaine. Can. Fed. Biol. Soc. Proc., 11, 78-79.

VOHRA, M. M. & REIFFENSTEIN, R. J. (1967). Comparison of adrenergic responses of rat and guinea pig vas deferens. Fedn. Proc., 26, 509.

WYLIE, D. W., ARCHER, S. & ARNOLD, A. (1960). Augmentation of pharmacological properties of catecholamines by O-methyl transferase inhibitors. J. Pharmac. exp. Ther., 130, 234-244.